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A quiet case for European science

WHATEVER issues the forthcoming election in Britain brings up, two things are reasonably certain: that the nation's place in the European Economic Community will be widely discussed and that science and technology policy will not. But, argues André Danzin in a newly published book *Science and the Second Renaissance of Europe* (Pergamon), the future of Europe is so much bound up in science and technology that if the public were kept adequately informed "scientific and technical policy would be expressed in terms of electoral concern".

What are we to make of Western Europe? Danzin, who is chairman of the European Committee for Research and Development (CERD) an advisory committee of twenty-one individuals, paints a very realistic picture of the deep problems, but also the immense potential of the region. The real resource is people and their cultural identity, but in recent years, limitations on space, materials, energy and money have dimmed the spirit. By almost any criterion, the nine of the EEC have failed to keep pace with the United States or Japan. (Danzin is excessively scrupulous about regarding the EEC as an entity, and almost never talks about individual nations; hence the economic and growing scientific strength of West Germany is neutralised by poor performances elsewhere in the community.)

He does not believe there is evidence that Europe is falling behind in its possession of knowledge; it is the "lack of concern" that is hobbling progress. And for Danzin, progress is undoubtedly closely tied for the foreseeable future to science and technology, and more specifically to the world of information. He shows a striking graph (admittedly from an American source) which demonstrates the colossal recent growth in numbers of those who could be said to be working in 'information' rather than agriculture, industry and personal services. It is estimated that more than 50% of the working population is now engaged in acquiring, storing, processing and transmitting information, or in working in allied support industries. And there is no sign that the trend which has lifted this figure from 15% to 50% in thirty years is yet over.

If there is every sign that information is becoming such an important commodity, and if there is no indication that Europeans are individually less well-endowed with intellectual capacity than, say, Americans or Japanese, why, Danzin asks, should Europe take the risk of being outstripped by its rivals "in the only specialised field where it is not seriously handicapped—brainwork and inventive capacity"? And yet R&D expenditure per capita in Europe is less than half that in the US, with signs of the gap widening; whilst in Japan (where financial comparisons are inappropriate owing to much lower unit costs of Japanese research scientists) the number of specialists working in

R&D as a fraction of the total population is double the European figure.

The troubles are not all simply in money and numbers of researchers, however. Danzin is highly critical of the sort of structures that individual European nations have evolved in the academic, bureaucratic and industrial world and which they now carry over into the machinery of the EEC itself. Rigidity, resistance to innovation, personal immobility; these are the constraints which prevent Europe responding flexibly to a changing world, and which contrast so unfavourably with American experience. For the businessman and industrialist the attraction of Europe ought to be, but as yet is not, the possibility of selling in a market larger than any other in the world. For the scientist and technologist it is the diversity of Europe which ought to be attractive—the many different traditions each bringing different skills and approaches to bear on problems. But this diversity can only be harnessed if there is an easy flow of people and ideas around Europe. With the exception of certain large projects, this cannot be said to be happening at present—if anything Europeans pair off with Americans rather than with fellow Europeans. Language is, of course, a barrier, and there is no sign (in Britain at least) of any improvement on this score. But it is more than language; there is much insularity of attitudes.

Danzin does not propose a crusade for the Europeanisation of science—his final chapter is entitled "Proposals for Mild Treatment". He notes that the research budget of the Community is only around 1% of the total for individual nations, but believes community-wide research has made encouraging progress, despite "pointlessly cumbersome" decision-making circuits. He would like to see the European research budget grow to a "substantial" level with a new executive body of more flexibility, able to take quick decisions and fund on a multi-annual basis. The new body would have not just to incorporate the existing European laboratories but also to stimulate them to take part in a "vast system of extra-mural exchanges".

Some will be disappointed that after such an astute analysis of the European problem, and after such a ringing declaration of the importance of science and technology, Danzin does not come up with some highly radical solutions. But this is the work of a realist; developments have to be evolutionary, and they have still to meet the approval of nine selfish governments, so proposals for a bonanza will get nowhere. On the other hand, a revolutionary change of attitudes costs nothing and can be effected almost overnight. A careful reading and re-reading of Danzin's quiet book by all of those concerned about the future of the EEC and its institutions would undoubtedly change a lot of minds. □



Recombinant DNA research: private actions raise public eyebrows

Rival scientific groups, often working towards common goals, share a close network of financial supporters.

David Dickson reports

LAST Thursday, the US Court of Customs and Patent Appeals confirmed its earlier decision that patent applications could be granted on two separate microorganisms, the one developed by scientists with General Electric, the other by Upjohn Co.

For the second time in 18 months, the court reversed a decision by the Patents and Trademarks Office refusing the patent applications on the grounds that the microorganisms were products of nature. The re-evaluation was made necessary by a Supreme Court ruling last June that the Appeals Court should reconsider its verdict in the light of a decision that a particular computer program was not patentable.

Three of the five Appeals Court judges, in repeating their previous position, said that they failed to see any connection between the computer program and the microorganism cases. And it could therefore be some time before a final verdict—the outcome of which is being awaited before patent applications are considered for a growing list of recombinant DNA inventions—is reached, since the Patent Office may well choose to take the case back to the Supreme Court for final clarification.

Whatever the outcome, however, growing confidence in the US business community that the development of recombinant DNA technologies promises large profits has led to a steady flow of venture capital to support research. Noone has yet made very much money, but high commercial expectations have helped raise the value (on paper) of the five small private companies most deeply involved to a figure estimated at more than \$150 million.

In the rush for commercial pay-offs, however, some of the tactics used are beginning to raise various concerns on Capitol Hill, such as whether private industry's voluntary compliance with the National Institutes of Health's guidelines provides an adequate basis for ensuring the same degree of safety in private industry as the guidelines currently require in university laboratories.

Some of the issues are:

- Private corporations have already financed research by US scientists in various European laboratories to carry out experiments for which the facilities required by the guidelines at the time were not available in the US.

- A major pharmaceutical company is providing backing for two of the leading three teams investigating the possible production of human insulin by bacteria (and has tried—unsuccessfully—to gain a license to develop the results of the third team).

- A transnational recombinant DNA company has been set up by a Canadian multinational company with a group of leading scientists from Europe and the US. Capital for the company has been raised on both sides of the Atlantic, and the arrangement permits experiments to be located in the country offering the most acceptable environment for the research.

- As reported in *Nature* last week, at least one US company is already planning to conduct experiments involving more than ten litres of culture without receiving prior formal approval from the NIH, as the current guidelines require of federally-sponsored research.

The rapid growth of the small research companies, representing a direct marriage between university research workers and finance raised on the venture capital market, bears many similarities to the growth of the electronics industry ten years ago. Although most of the large pharmaceutical companies are building up their in-house capability to do such research, in most cases they have been less aggressive and slower to accept the commercial potential of the new technology than outside investors who are more prepared to take speculative risks.

In addition, rather than selling the licence to develop patents arising from their research direct to the major

companies, a number of scientists have preferred to set up their own commercial ventures, often using the money they have raised to licence the patents from work they have carried out on federally funded grants. "A fact of life is that the vast majority of talent in the field is in the universities at the present time," says Professor David Jackson of the University of Michigan.

Five relatively small companies currently occupy the centre of the stage. These are:

- Cetus Corporation, set up in Berkeley, California in 1971 on an initial capital of \$5 million, and currently carrying out research on a contract basis for industry in a number of areas, including biomedical, chemical, food and agricultural technologies. Cetus has opened a P3 facility, and its current value on paper is estimated by president Dr Peter Farley as around \$65 million. Almost half of this stock is owned by Standard Oil and National Distillers Company.

- Genentech, Cetus' rival across the bay in San Francisco, which claims to be "the leading company in the application of recombinant DNA techniques to problems in human health care". Set up in 1976 by Robert Swanson and Dr Herbert Boyer, Genentech is also interested in a wide range of applications, but has gained most publicity for its work on somatostatin and insulin.

- Biogen, a company incorporated in Luxembourg, which was set up last year by Mr Dan Adams, then head of the venture capital division of International Nickel. Biogen has nine scientist co-founders from both sides of the Atlantic, and has so far concentrated mainly on working towards pharmaceutical products such as insulin, interferon and a possible hepatitis vaccine. Adams estimates the current value to be about \$50 million and Biogen is currently constructing laboratories in Switzerland to P3 containment levels.

- Genex, established in 1977 by Dr Jackson of Michigan and two others. It is concentrating on industrial processes involved in, for example, the food and chemical industries. Genex plans to open a laboratory in Rockville, Maryland in the near future, and to announce a major deal with "one of the fortune 500 companies", within the next four weeks according to president Dr Leslie Glick.

- Bethesda Research Laboratories, set up three years ago and already a major



supplier of restriction enzymes. It started moving into recombinant DNA research last summer, and has opened a P2 facility also in Rockville (rapidly becoming the "route 128" of molecular biology, largely because of its proximity to NIH).

In addition, collaborative research, in Waltham, Massachusetts, which already supplies materials such as synthetic genes required for research to a number of the companies listed above, is now contemplating entering the field directly, according to its president, Dr Orrie Friedman.

At the scientific level, research groups supported by the various companies are frequently exploring different paths towards the same goals, characterised by the fierce—though usually friendly—rivalry common to all fields of science. Some of these goals include the production by bacterial means of insulin, interferon, growth hormone, and various industrial enzymes.

At the financial level, however, the situation is slightly different. Apart from Bethesda Research Labs, which is financed by a private family trust, the other four companies share some common financial backers in what one participant acknowledges to be a "somewhat incestuous" relationship.

For example, Kleiner and Perkins, an established West coast venture capital firm which supplied the backing for electronics companies such as Fairchild Industries, has provided substantial support for both Cetus and Genentech, although Dr Farley of Cetus says that the standard oil support now puts the company apart from the other three. Similarly, New Jersey, which receives its own funds from companies such as Aetna Life Insurance, Emerson Electric, and the Monsanto Chemical Company, has a stake in Genentech, and last year provided a substantial amount of capital to Genex. Mr Gerald Large, president of Innoven, is now a director of Genex.

Perhaps the most extensive involvement has been that of International Nickel, (INCO), a Canadian based mineral company, which set up a venture capital division operating from New York in 1975 to support "worthwhile entrepreneurial investment opportunities in Canada, the US and Europe." Soon after it began operations, the then head of the division, Mr Dan Adams, selected recombinant DNA technology as a fruitful field for investment. "I felt that this was the most exciting field of technology for investment for the immediate future," he said last week.

In line with this conviction, INCO bought a small amount of stock in Cetus in 1976 valued at about \$500,000

and subsequently in 1977 bought a larger, 10% share in Genentech. Then in 1978, with advice from Genentech—which is now demanding a share in the equity in return), and a scientific board brought together by Dr Walter Philip Sharp of MIT, INCO set up a Gilbert of Harvard University and Dr new company, Biogen, with Adams as president (Adams has since left both INCO and Biogen, but is looking for new areas to start a similar venture).

INCO was incorporated in Luxembourg, "which has some attractive tax advantages", according to Adams, and sponsors research in the laboratories of a number of members of its scientific boards, such as that of Dr Charles Weissman, of the Institute for Molecular Biology at the University of Zurich.

Financing provided largely by INCO through Biogen has already allowed Gilbert to carry out experiments into the production of human insulin at the Microbiological Research Establishment at Porton Down in the UK, since at the time the P4 containment facilities required by the NIH guidelines were not available in the US.

Of the major pharmaceutical companies, Eli Lilly has been the most aggressive in pursuing potentially valuable research results (as well as the most vocal critic of attempts to legislate basic research in the private sector). The company currently controls 80% of the \$140 million domestic insulin market, and is keen to maintain this position.

Last Autumn, Eli Lilly entered an agreement with Genentech clinched soon after scientists in the latter company announced they had produced human insulin from bacteria (although the insulin produced in this way has yet to be shown to be biologically active) under which the two companies will carry out a joint long-term manufacturing and marketing programme.

In addition, Eli Lilly has entered a separate agreement with Dr Howard Goodman and Dr Bill Rutter of the University of San Francisco in the Department of Biochemistry and Biophysics. The pharmaceutical company has provided over \$250,000 to support the laboratory's research into the production of human insulin, money which last year allowed research workers from the laboratory to carry out experiments in France which could not be done in the US.

In return for providing financial support, the company expects first refusal on the license to develop any patentable results which arise from the research.

In addition to the above two agreements, Eli Lilly recently bid unsuccessfully to purchase from Harvard University the rights to develop the patent filed by Gilbert for research

developed in connection with other research teams at Harvard (the British drug company Boots, also expressed interest in licensing the patent but Harvard decided that the patent should go to Biogen).

Within this complex of financial and corporate interests, those involved are taking pains to emphasise that all research—at least that which is carried out in the US, and at least with respect to the technical aspects of containment levels—is conducted in accordance with the criteria in the NIH guidelines.

For research outside the US, however, it is generally accepted—even by NIH itself—that these guidelines need not apply, strictly but that the research should be done in compliance with local requirements (making countries with relatively low requirements attractive).

However, evidence that companies are already more than willing to move abroad to carry out research that cannot be done in the US is beginning to worry US lawmakers, which have so far held back from legislating over the private sector. "This is certainly something we are keeping a close eye on" a staff aide to Senator Adlai Stevenson's science subcommittee said last week.

In return, companies are worried about a proposal by the Food and Drug Administration to introduce regulations that would require all research leading to products for which licenses are applied to have been conducted under the NIH guidelines. They are suggesting that, if the work is carried out abroad, the compliance should be with local guidelines. "It would be silly for FDA or NIH to try to police the world" said Adams.

Meanwhile other critics of existing policy and of the NIH sponsored decision processes are concerned that without legislation explicitly covering the private sector, there will be no way of guaranteeing the safety of the procedures that this sector adopts. In the wake of last week's nuclear power incident at Harrisburg public credibility of claims of minimal danger is running low (already scientists at Tufts University have demonstrated that a "disabled" E. Coli strain classified as EK2 is capable of surviving up to 90 hours in the human gut, far longer than was initially anticipated).

"The question remains who is going to control both the process and the products of this research," says Dr Susan Wright of the University of Michigan. "Given that great uncertainties still exist, and the fact that risk assessment experiments have not yielded uniformly negative results and have indicated some areas for renewed concern, it is important that Congress does all that it can to guarantee the safety of those involved." □

COGENE plays up benefits, plays down risks

IN A surprise statement last Sunday, the foreign secretary of the Royal Society, Mr Michael Stoker, announced the lifting of the ban on reporting of what promises to be the most important meeting on the risks and benefits of recombinant DNA techniques since Asilomar, the conference held in California in 1975 during which scientists called for strict control of the use of recombinant DNA techniques.

COGENE, the Committee on Genetic Experimentation of the International Council of Scientific Unions (ICSU), had previously issued a directive that the whole proceedings at Wye College, Ashford, Kent, UK would be off the record. The directive was the result of what was felt to be unbalanced press coverage concerning recombinant DNA risks, particularly after Asilomar.

Dr Stoker, however, when opening the meeting, asked participants to

"tear up that note". There had, he said, been a change of attitude on the organising committee. But the note had already had its effect—only three journalists were present and they were from specialist scientific magazines, including *Nature*.

We are therefore now able to report that COGENE is shifting its attention from risks to benefits. Supported by UNESCO and the World Health Organisation, it is planning to hold a series of two to three week teaching courses in recombinant DNA techniques in developing countries beginning in January 1980. COGENE chairman Dr Bill Whelan said: "We have the money and we just want to find a country that would appreciate our help." Recombinant DNA techniques could be of great benefit in Third World countries, Whelan believes, particularly in agriculture, where they could help in a rapid assessment of the

molecular genetics of local crops.

Meanwhile, however, COGENE is slowing up its attempts to assess the risks of the technology. "We have run out of good ideas for risk assessment experiments", said Dr Anna-Marie Skalka, who chairs the COGENE subcommittee on risk assessment. However, Professor Mark Richmond, a member of the UK's Genetic Manipulation Advisory Group (GMAG) who will report on his own assessment experiments at the meeting, believes that the real problem is finding willing researchers to do the work. "I took it up to show willing", he said, but the work takes up time, lab space and people especially when there is a wide feeling in the scientific community that the risks are minimal. "If the UK Medical Research Council wants people to assess risks it may have to force them to do it", he said.

Robert Walgate

GMAG states the position on 'self-closing'

AT the end of last week, GMAG issued a statement designed to satisfy the "urgent need to clarify certain issues" and to remove "any uncertainty of ambiguity about present requirements". As expected some self-cloning experiments—those using *Escherichia coli* K12 and other well-characterised non-pathogenic laboratory strains of *E. coli*, *Bacillus subtilis*, *Bacillus stearothermophilus* and *Saccharomyces cerevisiae*—are exempted from statutory notification.

In general, however, self-cloning experiments are still considered to be within GMAG's remit. "The group is satisfied" says the statement "that many self-cloning experiments are without conceivable hazard", but it "interprets the definition of genetic manipulation to cover self-cloning experiments". It also acknowledges "that there has been uncertainty on this point and that some users have interpreted the definition to exclude such experiments". The definition of genetic manipulation used by GMAG is "the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced, by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation".

In future, therefore, all self-cloning experiments with systems other than those specifically designated as exempt, should be notified to GMAG which "will advise on containment". Those working with exempt organisms are

asked to provide voluntarily "a limited amount of information about their plans to help (GMAG) keep in touch with all developments in the field". This should include "species and strains, the place of work and the scientists responsible".

GMAG is also seeking to amend regulations to cover the use of "products of genetic manipulation which are capable of self-propagation". Voluntary notification of the use of these products is requested so that GMAG can advise on containment. However, exemptions from this will include the products of those self-cloning experiments which are exempt and the insertion of *Xenopus* genes coding for 28S, 18S and 5S ribosomal RNAs into prokaryotic DNA. Users are invited to propose other products of recombinants which could be added to this list. Special consideration is also to be given to scaled up work involving the growth of self-propagating products in volumes of 10 litres or more. Arrangements for this type of work are still under discussion.

GMAG still feels unable, according to the statement, to accept some of the principles that the United States and other countries have adopted, following the NIH lead. Nevertheless it will study the NIH guidelines to see if there are some procedures which could be adopted in the UK in the hope that collaboration with other national authorities will bring about some uniformity of procedure.

GMAG's terms of reference are restated as advising all those concerned in any way with genetic manipulation,

continually assessing new genetic manipulation techniques (and new containment methods) and advising on appropriate action. It is also required to "maintain appropriate contacts with relevant government departments, the Health and Safety Executive and the Dangerous Pathogens Advisory Group", and to keep records of containment facilities and the qualifications of biological safety officers. □

UK minister criticises COGENE meeting

Mr David Ennals, UK Secretary of State for Health and Social Security, expressed his concern last week at the original intention to ban reporting of this week's COGENE meeting. "Basically, I'm opposed to secret conferences", he told the genetic engineering subcommittee of the House of Commons Select Committee on Science and Technology. "Far too much in the field of science is done behind closed doors".

The subcommittee was holding its first public session after a recent visit to the US. Mr Ted Leadbitter cited a recent US survey which had found that of 18 countries using recombinant DNA techniques, the US and the UK had the most stringent regulations. Might this encourage valuable work to go abroad, he asked? Mr Ennals said he thought the most important thing was to "watch the hazards carefully" if we did not want to upset the balance between risk and benefit. GMAG he thought was flexible enough to do this. □

European physicists discuss the "great projects"

A SELECT group of European physicists met in Rome last week to learn of Europe's plans for future "very large capital projects" in astrophysics, sub-nuclear physics, synchrotron radiation and nuclear fusion—projects which, the organisers say, could dominate research and development until the end of the century. They were there by invitation of the European Physical Society, whose president, Antonino Zichichi, had decided that the meeting was needed to inform key physicists with an interest in any one of the projects of the plans for the others. All the projects are likely to compete, at least to some extent, for future funds.

Professor L. Woltjer of the European Southern Observatory (ESO) headquarters in Geneva, told the meeting that after the present generation of ground-based single dish telescopes of diameter 3-6m, the future of Europe's optical astronomy will rest with the joint NASA/ESA Space Telescope, due for launch from the Space Shuttle in 1984, and a ground-based Very Large Telescope (VLT).

So far the latter is only an idea but it is being considered seriously by the ESO. The current plan is that it would have a diameter of about 16m, would probably be sited at the ESO's site in Chile and would become operational in 1990: its cost is estimated at about \$100 million. As it is unlikely that a single dish of 16m diameter could be built, the VLT would probably be a multi-mirror tele-

scope.

The Space Telescope and the VLT will be complementary, according to Professor Woltjer. The former will be used for gathering data on very faint sources which could never be observed by a ground-based telescope because of the masking effect of the Earth's atmosphere. However, because it is relatively small (2.4m diameter), the Space Telescope will be limited in the number of photons it can collect. The VLT with its large collecting area, will be capable of collecting many more photons and will therefore be suitable for detailed spectroscopic analysis of relatively bright sources.

In radio astronomy, Europe is already active in very long base interferometry (VLBI), the aim of which is to produce a two-dimensional map of radio sources. There are plans to extend the VLBI network by building more receivers and also by using one of the European Space Agency's future communications satellites as a reference point for phase and amplitude information. The French are looking at the possibility of using VLBI at optical wavelengths. A major problem however, is that the engineering tolerances of the receivers have to be very high because of the small wavelength.

Fusion scientists are also planning their next move. According to Professor Palumbo from Brussels, an international team met last January to start discussing the machine to follow the Joint European Torus (JET) which is

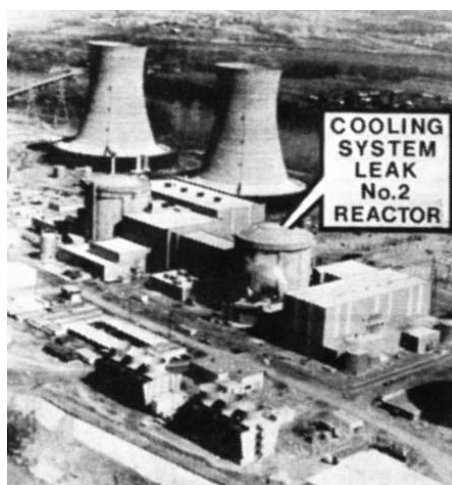
just getting underway at the Culham Laboratory in the UK. Just the week before the meeting, he said, the EEC had increased the budget for European fusion research by 20 million accounting units.

Two plans for major future particle accelerators were discussed. One is Europe's plan to build a large electron-positron storage ring (LEP) (see *Nature* 12 October 1978, page 469 and 14 December 1978, page 658), the other is the Soviet Union's plan to build a 3 TeV proton synchrotron. Work on the latter has been going on for "a long time" according to Professor Siderov of Novosibirsk who presented the project instead of Professor K. P. Myznikov of Moscow who did not attend the meeting. He could not say when the machine would be built or how much it would cost, but seemed confident that it would be built at some time. Professor M. Vivargent of CERN said that the design for LEP currently favoured would cost about SwFr 1000 million and should be built by 1988. More should be known at the end of this year, however, when the report of the latest study group would be ready.

Like LEP, the European Synchrotron Radiation Facility (ESRF), is also awaiting the publication of a report at the end of this year which should also lay down the type of machine the physicists would like. It will then be up to the politicians to decide whether or not that is economically feasible. **Judy Redfearn**

Harrisburg leak 'the worst accident in nuclear history'

THE type of human and mechanical failure which occurred in the nuclear power plant near Harrisburg, Pennsylvania last week and led to the discharge of a cloud of radioactive gas into the surrounding environment, has a 50:50 chance of happening in 400 reactor years, according to Dr Chauncey Starr, Vice-President of the US Electrical Power Research Institute. Speaking at a press conference earlier this week, Dr Starr said that "on the technical side this accident, while no-one wanted it, has a statistical probability that falls within the predicted probability of this type of incident. We have had 400 reactor years of operation of nuclear power and the earliest statistical analyses indicated that low level releases of radiation due to equipment failure would have this kind of statistical probability".



The accident resulted from an overheating of the core of the reactor causing some damage to the fuel rods. The emergency cooling system had flooded the core with water, but the contaminated water, some of which escaped through the roof of the plant as steam, was largely responsible for

the escape of fission products such as xenon, krypton and iodine into the local environment.

A critical problem still to be resolved is a 1,000 cubic ft hydrogen bubble trapped in the reactor core. Efforts to redissolve the bubble have failed and the radiation levels inside the plant are too high to release the pressure manually.

After a second release last Saturday a reading of 1,200 mrem per hour was taken at the gas vent, 25 mrem per hour south of the plant site and 15 mrem per hour within a five mile radius. Street and doorstep measurements in Harrisburg ranged from 2 to 5 mrem per hour. A normal chest X-ray produces 30 mrem of ionising radiation. Thus the inhabitants of Harrisburg, 10 miles from the plant are receiving the equivalent of from one to five chest X-rays per day with plant officials predicting several more days of radiation leaks. The possibility still remains that 600,000 people in a 20-mile radius around the plant may have to be evacuated. □

Finland turns to wood and peat for energy

ONE of the last acts of Finland's outgoing government was to accept, on 15 March, the report of a special commission on energy resources, which advocates 40% self-sufficiency by 1990. This programme, which will entail increased use of peat and wood, is effectively a reversal of a previous policy which envisaged a decrease of self-sufficiency from 29% in 1977 to 23% in 1990.

Since Finland's wood processing industry consumes approximately one quarter of all its energy and one third of its electricity, it would seem ecologically appropriate that it provide some of its energy needs. This it already does by providing some electricity generating plants (which in 1977 produced 7.5 TWh of electricity) with fuel in the form of wood wastes. Finland's Centrist Party, however, would like to go further than the commission's proposals and is suggesting an ultimate energy self-sufficiency of 50%. It says that proper use of wood wastes could replace up to 30% of Finland's oil imports. It is also advocating the growing of 'energy woods'—willow and other non-commercial timber. Finland's Forestry Research Institute is at present researching into this possibility, which would have the advantage of using otherwise unprofitable marginal and scrub lands. The main problem however is finance since planting and management of such 'energy woods' is highly labour intensive.

According to the 1977 forecasts, Finland's annual consumption of primary energy sources was expected to rise from 23.0 to 33.2 Mtoe by 1990—an increase of some 44%. Of these totals, oil consumption was expected to rise from 12.2 Mtoe in 1977 to 15.9 Mtoe by 1990. In 1977, approximately 70% of Finland's oil imports came from the Soviet Union, and it was hoped that the increase would also come from there.

Recently, however, the Soviet Union has been unable to meet Finland's increased demands, apparently due to similar increases from domestic and Comecon consumers, and difficulties in bringing the new Siberian fields on-stream. At the same time, OPEC prices have continued to rise, and the Soviet Union raised its prices proportionally—14% last December, and a further 8–9% last week.

Furthermore, Finland's two supertankers are not allowed to pass through the narrows between Denmark and Sweden if loaded to their full 100,000 tonne capacity. A major rethinking of energy policy seemed timely.



Making a grab for home-grown timber

Being a relatively flat country, Finland has only a limited number of sites suitable for hydroelectric plants, and these are already exploited virtually to full capacity (12.1 TWh in 1977). Nuclear power at present provides some 7% of all generating capacity from the first set at the Loviisa power station. A second set is under construction at Loviisa and two sets at Okiluto. The possibility of a third nuclear power station with a total capacity of 1 GW is still under discussion, but no decision is expected until the early 1980s.

Finland, incidentally, does not seem to have a vocal anti-nuclear lobby. However, on accepting the Soviet tender for the Loviisa plant, the Finns demanded far more stringent safety precautions than were then normal for Soviet reactors, including containment buildings (seen by the Soviet designers as a capitalist trick for inflating construction costs), redundant cooling systems and improved computerised controls. When the Soviet Union was unable to supply these additions, the Finns went shopping in Holland, West Germany and the USA.

Nuclear power, however, depends on foreign suppliers, not only for the construction of the station (the Soviet Union for Loviisa and Sweden for Okiluto) but also for nuclear fuel. If Finland is to become relatively more self-sufficient, wood and peat will become considerably more important.

Until very recently, peat which at present accounts for only 1% of Finland's energy production, had not been considered a commercially viable alternative. Earlier this month, however, the then Minister of Trade and Industry, E. Rantala announced a new agreement between Neste Oy, the Imatra Power company and the Navire Company on the study and adoption of wet carbonisation. This process removes most of the water from the peat, giving a fuel that is comparable with coal and contains only some 10% of water. Liquefaction of peat, said Mr Rantala, is also to be studied and the next supplementary budget is expected to allot additional funds for peat research.

Vera Rich

Hungary's careful use of pesticides for plant protection

"CHEMICAL pesticides, even if used extensively, never harm the wild life economy, provided that they are applied professionally." So said Mr Ferens Hargetai, head of the plant protection department of the Hungarian Ministry of Agriculture, at a seminar in Cambridge, England last week.

Hargetai's statement might well alarm some ecologists, particularly when joined to his definition of environmental protection. By this, he said, "we mean, whether confessed or not, the human being rather than the environment itself. Speaking about environment protection, one never thinks of the protection of the Colorado beetle or house fly, though these also belong to the environment in the same way as the singing birds or the national parks do".

However Dr Illes Desi, of the National Institute of Hygiene, suggested at the seminar, that developing an appropriate pesticide strategy was by no means simple. "More pesticides", he told *Nature*, "mean more food—but also more pollution."

The Cambridge seminar was part of the UK-Hungary trade and technology cooperation programme, and it was the Hungarians who asked specifically for a meeting on pesticides. Hungary has an excellent record of awareness of pesticide hazard. Mr Hargetai said it was the first country in the world to ban DDT, aldrin, dieldrin and HCH. "We found that the concentration of DDT in the population was very high—12 to 16 ppm. This was among the highest in the world. So it had to be stopped".

In the same year (1968), a set of requirements for the registration of pesticides was drawn up. In 1972, a new system was introduced classifying pesticides from the point of view of environmental hazard. This meant certain pesticides could not be used within a given distance of a river; some may only be applied by trained experts.

These experts play an important role in Hungarian agricultural planning. By law, every state farm must have on its staff a "plant protection engineer". Cooperatives and the few remaining private farms can (and in the case of dangerous pesticides, must) hire the relevant experts from the government-sponsored Plant Protection Service.

This service is concerned with the whole range of plant management problems including research into crop pests and diseases. In 1975 a forecasting system began which has reduced considerably the amount of pesticide needed. □

news in brief

Breakthrough by PLUTO: The PLUTO collaboration, working on the e^+e^- storage ring at DESY in Hamburg, announced last week the discovery of new evidence to lend support to Quantum Chromodynamics (QCD) as the proper theory for strongly interacting particles. Recent data at DESY shows evidence for a three-jet structure in the decay of the u quark just as predicted by some QCD models. QCD model calculations for e^+e^- collisions show that the u quark decays into 3 gluons which give rise to 3 jets of high momentum particles. The identification of such multi-jet events is very difficult because of severe backgrounds from other processes. The PLUTO analysis is based on a sample of over 2,000 events collected in the u quark resonance region. Earlier studies of such jet structures have been inconclusive. Only after many refinements of its data analysis procedures has the group been able to confirm the existence of 3-gluon jets.

Boost for biotechnology: A UK working party on biotechnology is being set up to review developments in this rapidly expanding field and to recommend actions which will help British industry. The sponsors of the working party are the Advisory Council for Applied Research, the Advisory Board for the Research Councils and the Royal Society. The chairman will be Dr A. Spinks of ICI. The group is likely to take a very wide look at the subject in disciplines such as photosynthesis, recombinant DNA, fermentation biomass, cell fusion and so on; applications are likely to range through pharmaceuticals, waste disposal, food technology, metal mining, oil production and many others.

Health check on UK radiation workers: The Medical Research Council (MRC) is to conduct epidemiological studies for the UK Atomic Energy Authority (UKAEA) to investigate the effects of long term exposure to low levels of radiation on UKAEA workers. The research team will probably survey the health records of all present and past UKAEA workers and visit all its institutions. Workers employed by British Nuclear Fuels Limited (BNFL) will not be included in the survey as BNFL are carrying out an independent survey. According to Dr Tony Vickers of MRC Headquarters, the head of the study has still to be appointed and results will not be published for at least three years. The whole survey will be monitored by a steering sub-committee of MRC's Protection against Ionising Radiation Committee, chaired by Dr R. H. Mole, a former director of Harwell's Radiobiology unit.

FOE requests reactor safety information: In the wake of the Harrisburg accident (see page 497) Friends of the Earth has renewed its request to the UK Secretary of State for Energy, Tony Benn, to publish the Health and Safety Executive's full report on pressurised water reactor safety. Citing the recent disclosure of the Union of Concerned Scientists "nugget file" of "astonishing" safety deficiencies at US power stations, the closure of five power stations due to a factor of six deficiency in the design of safety piping and the continuing Harrisburg events, FOE criticises the HSE for refusing to release detailed safety studies of PWRs or of the British Gas Cooled Reactor (AGR). In addition, FOE requests Benn to submit the full reports to "a proper review process" by independent analysts.

Meanwhile, British Nuclear Fuels has announced that the latest Windscale leak (29 March) has been located in an out-of-use building that had been used to store highly active wastes before their transfer to buffer storage tanks. BNFL is now investigating the route by which the leak

reached the borehole and is satisfied that "there is no hazard to workers on the site or to people in the nearby community". The radioactivity measured in the borehole was 0.1 curie per litre. It was not possible to obtain the energy of the radiation for conversion to dosage (rems).

US urged to centralise assessment of cancer risks: A single scientific organisation responsible for identifying and characterising potential carcinogens across a range of government agencies has been recommended in a report prepared by the staff of the US Office of Science and Technology Policy. Such an organisation, which it is suggested should be based on an expansion of the National Toxicology Programme established last year by the Department of Health, Education and Welfare, would have two main responsibilities: to identify those substances which pose a potential carcinogenic risk to humans, and to characterise in quantitative terms the nature of the risk and the degree of certainty of current estimates.

Priority for scientific analysis should be given to chemicals for which cost-benefit analysis performed with existing data suggests that regulation be indicated, but leaves too much uncertainty to justify immediate action. "Priority setting and coordination of testing ideally should be done by a single group with input from all relevant agencies" says the report.

Environmentalists criticise secrecy of recombinant DNA meeting: Three US environmental groups have issued a statement criticising this week's meeting on recombinant DNA research organised by the Royal Society and COGENE for placing a cloak of secrecy around its deliberations. The meeting, participants of which had been told that they should not report directly on the proceedings, has been discussing the development of guidelines by different countries to regulate this research. Leslie Dach, of the Environmental Defense Fund, said that "it is a disservice to the discipline of science that the press and the public be excluded from a meeting that is concerned with a new technology of potential global impact." (The ban on reporting was suddenly lifted at the start of the meeting, see page 496).

EDF, Friends of the Earth and the Coalition for Responsible Genetic Research have claimed that the \$200 attendance fee is a barrier to the public against attending the conference. "An international group such as UNESCO cannot afford to condone exclusion of the news media and other observers from an issue as important and debatable as genetic engineering," said Lorna Salzman of Friends of the Earth (COGENE is organised under the auspices of the International Council of Scientific Unions, which is supported by UNESCO).

US study claims benefits of reducing air pollution outweigh costs: The US Environmental Protection Agency has issued a report saying that the costs of cleaning up air pollution from factory chimneys is considerably less than the economic benefits including the reduction in health expenses. According to the EPA, the annual health spending of the US would be reduced by \$40,000 million if pollution from factories was reduced by 60%, although this figure has already been queried by the business community. The report was prepared by research workers at the University of Wyoming, the University of New Mexico and the University of Southern California. It stated that a 30% improvement in the air quality of the Los Angeles region would produce an annual benefit of \$500 per household by raising real estate values.

Swiss go to polls again on nuclear issue

Local democracy continues to block nuclear power in Switzerland. Geoff Milnes explains the latest debate over waste disposal

THE further development of nuclear power in Switzerland is grinding to a halt because of the refusal of local authorities to allow test borings in the search for suitable repository sites for radioactive waste. This is for many people an unwelcome effect of direct democracy and local autonomy—two characteristics of political life in Switzerland of which the country is justly proud. Local resistance to test borings has led to a polarisation which has spread to the whole question of the role of nuclear energy. This became clear in the campaign leading up to the plebiscite on 18 February, in which 49% of the voters were in favour of a ruling which would probably have brought nuclear development to a complete halt, by demanding popular control of the siting of nuclear facilities by the local population surrounding the proposed sites.

Moreover the situation will remain critical if a revision of the law which recently passed through parliament is turned down in the next referendum, in May.

The present law governing nuclear power plants was put into force in 1959 with the idea of encouraging the peaceful use of atomic energy by the electricity companies, which are mixed

private and cantonal bodies run on a private enterprise basis. Licensing was placed in the hands of the *Bundesrat* (the seven-man cabinet) with no direct democratic or parliamentary control. There was little controversy until mid-1974 when newspapers reported surreptitious drilling by a little-known organisation, NAGRA, looking for repository sites for radioactive waste in a number of localities—without the permission of the local authorities.

This action, together with the obvious and painful dependence of Switzerland on foreign energy supplies emphasised by the oil crisis of 1973/74, a series of frightening projections of future energy requirements, and plans for building 10-15 nuclear power stations which appeared at about the same time, resulted in a tremendous upsurge of public interest and concern.

NAGRA (Nationale Genossenschaft für die Lagerung Radioaktiver Abfälle) is a cooperative organisation founded in 1972 by the "waste producers"—the companies operating or planning to operate nuclear power stations (six companies in all) and the confederal government (responsible for radioactive wastes derived from hospitals, industry and research)—each with one delegate on the administrative board.

This board contracts research in various directions, financed in proportion by the companies and the government—in waste treatment and conditioning, in the development of repository concepts, and in geological studies. The latter has turned out to be the politically critical area.

Switzerland is one of the few countries without a geological survey—and there is no tradition of free flow of geological data, since most studies are carried out by private companies in competition with each other. The NAGRA research was thus contracted out to a private geological bureau. Also, NAGRA became a member of a further institution, KUS (Konsortium für Untertagsspeicher), set up to coordinate all work on underground storage, including the storage of oil and natural gas. In 1974, none of these bodies were in the public eye and the complicated inter-relationships made it difficult for the individual landowners to grasp for what and for whom the geological investigations, including test drilling, were to be carried out, when permission was being sought for land access.

Whether this confusion was a result of a basically nuclear legal situation (NAGRA's view) or whether it was the result of a deliberate policy of secrecy and stealth (the anti-nuclear front's opinion) is hard to judge.

What the geologists say about where to bury the waste

A RECENT book* assesses the alternatives for disposing of the radioactive waste from Switzerland's nuclear power plants, assuming that by the year 2000 a final capacity of $5 \times 1,000$ MWe will be reached. From the geological point of view, radioactive wastes can be put into two categories, according to the length of time that isolation from the biosphere is required.

The first category, low-active waste (NAA), consists of contaminated materials from the normal running of power stations (filter residues, worn-out parts, tools, clothing, etc) and the radioactive debris produced on demolition after the projected 40-year running life. This material contains only short-lived radio-nuclides in small amounts and needs isolation for a few hundred years. The total volume will probably be about $85,000 \text{ m}^3$.

The second category is the high-level α -bearing waste (HAA) which originates from the reprocessing of

the spent fuel. Switzerland depends on foreign plants for fuel reprocessing, but must assume that high-level wastes from these plants will be returned to the country for disposal after an initial storage period. With present reprocessing procedures, which seem unlikely to change drastically in the near future, HAA will contain quantities of plutonium and α -emitters with long half lives which require containment times of at least 100,000 years. However, the total amount of HAA will probably be small (about 400 m^3).

However, several factors make Switzerland far from ideal for the location of radioactive waste repositories, particularly for HAA. These include the complicated geological structure, the humid climate and the complex hydrogeological conditions, the possibility of renewed extensive glaciation and the continuing tectono-seismic activity.

Near-surface mined cavities for low level waste: NAGRA has been contracting research in this direction for a number of years. Research has concentrated on anhydrite as the host rock for NAA repositories. Pure

anhydrite is suitable for cavern construction and has good sorption characteristics and thermal conductivity. It is also generally impermeable to ground-water near the surface, because of a "self-healing" effect caused by its rapid conversion to gypsum (with a volume increase of 60%) when water percolates along cracks and fractures. Anhydrite-bearing formations are widespread in Switzerland, though occurrences of pure anhydrite of sufficient size have not yet been found. The anhydrite occurrences known are all very impure and heterogeneous, often chaotically mixed with other rocks or found along fracture zones with rapidly varying thickness. Investigation on other potential host formations (shale, granite) have not yet been carried out. So, although a reasonable solution can be expected in this direction, the research and development programme is still in its infancy.

Deep borehole repositories for high level waste: The book proposes a fanned array of deep boreholes, penetrating a 350 m thick host formation at a depth of $1-2\frac{1}{2}$ km. Various potential host rocks—rock salt, anhy-

Nevertheless, when the news broke that test borings in connection with the search for suitable underground repositories for nuclear wastes were in progress in several places without official knowledge or consent of the Gemeinde in question, a great cry went up.

Since 1974, no further test borings have been possible and the resolution of this stalemate has become a key issue in the nuclear power controversy. The core of the legal problem seems to be that the *Bundesrat*, under the law of 1959 which is still in force, is only responsible for site licences and permits once a site has been selected. NAGRA has always emphasised, however, that the test borings were exploratory, preceding site selection.

The *Bundesrat* has thus decided that they are governed by the normal laws applied to mining, search for oil, etc., for which the Canton is responsible, after consultation with the Gemeinde concerned. NAGRA lawyers maintain that the preliminary investigations must be considered as an integral part of the nuclear facilities themselves, coming under confederal law, and so the NAGRA put in applications for permits to carry out the borings to the Confederate Energy Office in 1976. These are still pending.

This is not the only legal bone of contention. The other important one concerns the licensing of the power stations themselves. The anti-nuclear

movement, supported by eminent lawyers, maintains that the proof of safety of nuclear power stations, a necessary condition for the granting of construction licences, includes also the safe disposal of the radioactive wastes, and that this cannot be produced prior to repository site selection, i.e. before test borings have been made.

This is just one of a series of irregularities which lead them to the conclusion that the nuclear plants now in operation (three, with a total capacity of 1,000 MWe) and the two 1000 MWe units at present under construction were illegally licensed. In the general atmosphere of public discontent, as illustrated by last month's vote, the *Bundesrat* has considered it politically inopportune to license further works under the old law (the Kaiseraugst works, whose site was occupied by demonstrators for 6 weeks in spring 1975, is one whose licence is still pending). Instead, it set a commission to work to revise it.

The new law, which passed through parliament in a number of turbulent sessions in spring 1978, contained several improvements and clarifications most of which intended to place the nuclear industry under stricter and more democratic control, with regulations which, in contrast to the old law, should rather discourage nuclear power growth unless absolutely necessary.

For the "borehole stalemate", however, the new law brings some relief—

test borings for power stations or waste repositories can be forced through by the confederal authorities by expropriation of land, if necessary. But the situation is still tense.

A section of the anti-nuclear movement decided that the stalemate suited their purposes well and have forced a referendum *against* the revision of the 1959 law (voting takes place on 20 May). In this, they have laid themselves open to accusations of illogical obstructionism by the nuclear power supporters—it is pointed out that one of the opponents' main arguments against nuclear power is that the problem of radioactive waste disposal is not solved, and yet their activities are directed towards preventing the very investigations necessary to reach a solution.

The anti-nuclear group is intent on preventing the waste disposal problem—which must be solved whether more nuclear plants are put into operation or not—from being approached in the scientific way it deserves. In so doing, it risks alienating that part of the anti-nuclear movement which takes a broader view of the whole energy problem and which is interested only in nuclear power being kept within carefully and democratically controlled limits. Be that as it may, the Swiss voters will be asked to decide another nuclear issue in May and an important public debate continues. □

Geoff Milnes is at the Geology Institute, ETH, Zurich.

drite, shale, marl, gneiss, granite—are possible, but few are thought to occur at convenient depths in sufficient thickness (probably only gneiss and granite). Potential sites are more likely to be found outside the mountain areas, i.e. under the Swiss Plain (Molasse basin). In the Alps, the deep structure is very complicated and difficult to predict and the danger of a new Ice Age and rapid glacial erosion in the next 100,000 years cannot be ignored.

A repository at 1–2½ km depth in a suitable area is unlikely to be affected by "external" effects—human influences, meteorite impact, volcanic eruption, glacial erosion—during that period. The relatively high seismic risk in some areas is a more serious problem because the fracturing associated with earthquake activity could affect the underground water circulation, which is critical for the integrity of any repository. Information about the numerous hot spring and mineral water occurrences indicate high permeabilities and rapid groundwater circulation in some deep formations. It will be necessary to gather detailed information on the flow types, velocities and chemistry of the

groundwater and on the sorption properties of the surrounding rocks in the vicinity of the repositories. These are local parameters, not predictable from surface studies—their investigation requires exploratory deep drilling and long-term, on-site experiments. This is a costly and time-consuming business and is by no means sure of success. Ten to 15 years of directed research will be required before the feasibility of this concept can be settled.

The alternative to the two concepts summarised above is cooperation in an international programme such as the "seabed" programme. Whether the two concepts can be realised also depends also on social and political aspects. The situation is aggravated by the lack of an established geological survey and because the research, being carried out mainly with private financial support in a piecemeal fashion, is not published and not made available to the general public. It seems clear, however, that even with the most favourable political developments, the scientific and technical problems are such that a practical solution and definitive site selection can hardly be expected



The Alps: their complicated structure may make them unsafe for long-term storage.

within the next decade, particularly for the HAA category.

**Geologische Aspekte der Endlagerung radioaktiver Abfälle in der Schweiz* (Bürgisser et al. 1979), Schweiz. Energie-Stiftung, Report Nr. 6.

correspondence

Energy unit for global use

SIR,—Very large powers of 10 occur in discussions of global energy, one example being the exajoule (10^{18} joule). As this number is larger than the number of seconds since the big bang ($\sim 5 \times 10^{17}$) its size is difficult to appreciate, and a more intuitive unit is desirable.

Annual energy consumption is dimensionally power, and a good unit of power is a 60W continuously burning light bulb. (One could make it a 100W bulb, but that is slightly less modest.)

Furthermore it makes sense to report power per capita of the world population. Table 1 then expresses some important energy flows: the figures for 2000 AD and 2020 AD are based on exhibit 19 of Bloodworth, I. J. *et al.*, World Energy Demand to 2020, Executive Summary (World Energy Conference, London, 1977).

The table shows the low average efficiency of photosynthesis: there is evidently scope for the development of high energy yielding crops.

To see the proposed unit in action, we ask to what extent the deserts of the world—a somewhat fuzzy concept—could supply world energy requirements by direct conversion of solar radiation (to electricity, say). This is not a practical idea at present, but it is nonetheless important to have answers to such a question.

Assuming rather poor devices of 1% efficiency and an insolation at one tenth of the solar constant, one finds that the deserts would give an adequate yield for some decades to come (Table 2). Although there is in fact a great deal in hand at present, this advantage is being eroded.

The numbers for the energy consumption are reasonably small when expressed in these units and it is hoped that they carry an intuitive meaning.

Yours faithfully,

P. T. LANDSBERG

Department of Mathematics,
University of Southampton, UK

Military structure is off-target for science jobs

SIR,—David Horrobin's military solution to the problems of job opportunities and job security (8 March, page 118) could more aptly be described as the final solution, for if implemented, his ideas would further encourage short-term grant funded work using basic scientists as technicians and assistants. The lack of prospects offered would simply discourage those wishing to enter research as a profession and would commit research to a supportive role in advancing the careers of teachers and, in medical research, clinicians. But what is the problem he is trying to solve? If it is the "unequivocal . . . decline in research productivity between the years of 35 and 45" then his efforts are misguided: I challenge the myth that research scientists are more susceptible to 'burning out' than any other professional group. The "decline in research productivity" reflects a combination of withdrawal of short-term funding support (experience comes expensive) and the scientist's disillusionment with the abysmal career prospects.

In his proposed career structure we would know by the age of 35-40 whether or not we were suitable. If not, the alternative would be "the comfortable knowledge that they would have three years . . . to plan a second career." Who on earth is going to employ a middle-aged scientist stigmatised by his so-called "decline in research productivity." Like the universities, industry and government recruit largely at the graduate level and so even if they wished to pick up the academic 'casualties' they would not be able to do so. These proposals would also curb any independent thought, for the research worker would be continuously trying to please and impress the departmental 'Field Marshal' in the hope of being awarded another five years in

the despatches for efforts above and beyond the call of duty. Lastly, he resorts to the expedient of the gratuity payment. These always seem very generous at the time but, as many workers have recently found, the money is very soon spent, leaving the dole queue and senescence as the only feature on a bleak horizon.

Certainly scientists need a stimulus to work. But this should take the form of improved career prospects with adequate peer review by which effort, achievement and responsibility are rewarded; the game of Russian roulette in which the trigger is pulled every five years is no basis for a profession.

Yours faithfully,

MICK KADLUBOWSKI

Association of Researchers in
Medical Sciences,
London, UK.

Sergei Kovalev: remember a colleague

SIR,—Soviet biologist Sergei Kovalev is now in the fourth year of a seven-year term in a strict regime labour camp. He was sentenced in December 1975 for making known various violations of human rights by the Soviet state. Past support by scientists around the world has helped him to receive needed medical assistance and has ameliorated the harsh punishment given to him in the labour camp. Renewed efforts are now required to support him, lest he fade from the public eye and be left to the "mercy" of his jailers.

Scientists at the State University College at Buffalo and State University of New York at Buffalo have contributed funds and have purchased a journal subscription in Kovalev's name. It is sent regularly to him at his prison address. Colleagues at other institutions might well do likewise. Letters to Soviet authorities may also be helpful.

As spring approaches most of us in the northern hemisphere are going about our research and teaching and sharing the love of our families. Let us remind ourselves of our colleague, Sergei Kovalev, who is freezing in a Siberian labour camp because he dared to speak out in favour of human rights. We owe him a great debt and should do all that we can to help him in his time of need. As a symbol of our concern I suggest that each of us send a letter or greeting card to Dr Kovalev at:

Pochtovy Yashchik
No 5110-1BC
Moskva
USSR

This will let him know that he is not forgotten and will remind Soviet authorities that we have not forgotten our colleague. A blizzard of greeting cards would be an appropriate message at the end of this winter season.

Yours faithfully,

JAMES R. SPOTILA

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Buffalo, NY, USA.

Table 1 Important energy flows (Earth averages)

	60W bulbs per capita	Joules per annum
1975 [World population 4×10^9]		
Food consumption	2	1.5×10^{19}
Energy consumption	33	2.5×10^{20}
Photosynthesis yield	410	3.1×10^{21}
Solar energy incident on Earth	396,000	3.0×10^{24}
2000 AD [World population 6×10^9]		
Energy consumption	51	5.8×10^{20}
2020 AD [World population 7×10^9]		
Energy consumption	76	1.0×10^{21}

Table 2 Possible power yield from deserts

Year	Yield (60W bulbs p.c.)	Requirements from Table 1 (60W bulbs p.c.)
1975	141	33
2000	94	51
2020	81	76

Mean insolation is 135 W m^{-2} ; efficiency of devices 1%.

news and views

Testing solar cells in space

from Andrew Holmes-Siedle

THE silicon photovoltaic cell was, as the Americans say, one of the first 'spin-offs from the space program'. There are now intensive development projects world-wide with the object of developing economical terrestrial photovoltaic power plants. At the same time, US scientists are still attempting to develop cells to give even higher efficiency in space. The problems faced in the latter work are worth mention for three reasons. First, the increased understanding of cell action, engendered by this work, will benefit all solar-cell designers; second, the problems faced represent interesting examples of the sophisticated engineering of semiconductor materials; third, some recent measurements of silicon cells, mounted on unmanned vehicles in space and monitored remotely, have yielded unexpectedly useful information in a different field, namely data on the characteristics of the near-earth radiation environment.

A paper by Richard Statler and Delores Walker of the US Naval Research Laboratories (in the *Proceedings of the International Energy Conversion Engineering Conference*, San Diego, August 1978) describes the design, exposure in orbit and interim

results of a solar-cell experiment in orbit at an altitude of 22,000 km. The satellite was the second Navigational Technology Satellite, NTS-2. This unmanned satellite is intended as a test-bed for new technology. It was launched on 23 June, 1977 and will be monitored for at least 3 years. The small array of cells, flown as an experiment, was independent of a large array of conventional cells which powered the vehicle. Various new types of cell were mounted in groups on the surface of the satellite and various thin, transparent cover glasses were applied in order to absorb the worst of the particle radiation. However, many energetic particles cannot be fully stopped. These penetrating particles degrade the performance of the cells. Significant information concerning the numbers of particles can be obtained from this degradation, as will be described later.

New techniques for increasing cell efficiency were being tested on this flight. For example, the texturing of the front surface of the silicon wafer allows more sunlight to be captured, the product is called a 'non-reflective' cell; very shallow n-on-p junctions give improved response to short-wavelength

sunlight and the product is called the 'violet' cell; the rear surface of the cell can be treated to repel current carriers and the product is called the 'back field' cell; the back surface of the cell can be polished to reflect penetrating infrared light back into the conversion region, the product is called the 'back reflector' cell; finally, solid-state processing of the silicon itself can improve the diffusion of current by removing any existing defects or by preventing the formation of point defects ('radiation damage') by particle radiation. The 'lithium-doped p/n' cell is an example of a radical modification of processing which has the sole object of reducing the sensitivity of the cell to radiation damage.

Four different manufacturers supplied a variety of developmental designs using the above techniques. The table lists the types of silicon cell flown, their photovoltaic properties and their degradation after 223 days in space.

At the top of this table come advanced designs from each of the major manufacturers of cells intended for use in space, showing that several alternative high-efficiency designs can survive well in space. The Solarex cells had excellent initial efficiency but suffered incidental electrode problems, so that no verdict can be passed on these. The only major difference between cells 6 and 9 was the technology for sticking on the cover glass for space solar arrays. This difference is far from trivial, since ultraviolet light from the Sun can seriously affect the adhesive. The use of a fluorocarbon polymer adhesive (on cell 6 only) gave rise to the lowest percentage degradation of any cell and this finding may represent a major step forward in space technology. If we wish to use the usual silicone adhesive, we are forced into the extra expense of depositing an ultraviolet-rejecting film on the cover-slide (compare cells 1 and 7). Cell 10, despite its low position on the absolute scale, represents a modest success for the older p-on-n junction technology. The structure is intrinsically more sensitive to radiation but the results

Table 1 Maximum power output of silicon solar cells after 223 days in orbit on NTS-2 (n-on-p junctions of area 4 cm² unless otherwise stated).

	$P_{\max}(t)$ (mW)	% Power lost in 223 days	Manufacturer	Type
1.	63.2	12.2	Comsat	Textured, non-reflecting (with ultraviolet filter)
2.	60.5	13.6	Spectrolab	Reflecting back surface
3.	57.6	13.5	OCLI	'Violet' cell
4.	53.9	15.5	Spectrolab	'Sculptured' surface
5.	52.9	12.7	Spectrolab	Main array cell type
6.	50.5	8.8	Spectrolab	'Sculptured' (with PTFE special adhesive)
7.	49.6	33.6	Comsat	Textured, non-reflecting (no ultraviolet filter)
8.	48.5	13.9	OCLI	Conventional cell type (silica cover with silicone adhesive)
9.	47.6	11.0	Spectrolab	'Sculptured' (with silicone adhesive)
10.	47.0	15.8	Spectrolab	p/n lithium-doped
11.	40.3	13.9	OCLI	Conventional cell type (glass cover with special bonding method (ES)).
12.	33.3	46.5	Solarex	Vertical junction

demonstrate in space the laboratory finding that lithium atoms incorporated in the lattice can migrate and inactivate the defects created by particle radiation (Faith *IEEE Trans. Nucl. Sci.* **NS-12**(6) 371; 1972). Without the presence of lithium, another 12% of degradation would have been experienced. As regards silicon cell technology in general, one can say that the table shows that, given the economic incentive, silicon technology can be made to give still further advances in performance. A cell made from AlGa-As also did well in this experiment.

The NTS-2 experiment gave some useful information concerning radiation fluxes in space. The environment of the Van Allen belt has been crudely characterised but controversy continues about the absolute values of particle fluxes in orbits such as those of NTS-2 (a circular orbit of altitude 20,190 km, with the plane inclined 63.5 degrees to the plane of the equator) and of geostationary communication satellites (circular, 35,863 km, inclination 0). The main problem lies in measuring the spectra and spatial spread of the high-energy electrons which are known to circulate in geomagnetic trapping regions between altitudes 500 and 40,000 km. It is also difficult to follow the fluctuations of these values with time over several years. At the same time, engineers designing spacecraft are urgently in need of more precise predictions as to how rapidly their devices will degrade in this hostile environment. Thus any information which we can glean from well-calibrated cells such as those on NTS-2 is very welcome, especially since, on this flight, two experimental, remote-reading radiation dosimeters were also flown (Evans *et al.* *IEEE Trans. Nucl. Sci.* **25**, 1619; 1978). Certain p-n diode structures degrade smoothly and predictably under irradiation. High-energy particles produce recombination centres in the silicon by atomic displacement and these centres cause a reduction in minority-carrier diffusion length, L . Photovoltaic efficiency is proportional to L^2 . Thus, some solar cells constitute good, if limited, integrating radiation detectors. Results such as those tabulated here can be used to reduce the uncertainty inherent in the current models of particle fluxes in the Van Allen Belt and predictions of the effects of these fluxes on other electronic devices. The NTS-2 experiment has helped to increase our confidence in the fluxes of electrons to be encountered in this orbit (an annual damage-equivalent flux of 2×10^{14} 1-MeV electrons cm^{-2} per year). This figure is six times higher than some of the estimates used previously, which were based on the crude belt models already mentioned.

The NTS experiments, though modest, demonstrate that it is advisable to test theoretical predictions of performance in space by flying a 'test-bed' vehicle. The experiments also show that useful information can be obtained by employing, as detectors,

certain semiconductor device structures which are not yet widely used in radiation physics. $\square$

A. Holmes-Siedle is a consultant to the Physics Department of the Falmer Research Institute, Slough, UK.

Have quarks been found?

from P. I. P. Kalmus

MORE evidence for the existence of fractional electric charge has been claimed in a recent paper by G. S. LaRue, W. M. Fairbank and J. D. Phillips of Stanford University (*Phys. Rev. Lett.* **42**, 142; 1979). Results from an improved version of their superconducting magnetic levitation experiment, indicate residual charges of $+\frac{1}{3}e$ on two small niobium spheres. These together with results presented two years ago by LaRue, Fairbank and A. F. Hebard (*Phys. Rev. Lett.* **38**, 1011; 1977), if accepted at face value, show a total of four fractional charges in a sample of 7×10^{-7} kg of niobium. If these results are verified they represent a major discovery in physics, the detection of free quarks.

In classic experiments starting in 1910, R. A. Millikan sprayed microscopic oil droplets into an air gap between two parallel horizontal plates. The drops were charged by friction or by X-ray ionisation. They normally fell under the influence of gravity, but their motion could be changed and even reversed by the application of an electric potential difference across the plates. The viscous drag of the air caused drops to acquire terminal velocities which were functions of their electric charge. Individual drops were observed for long periods and their velocities determined. From many such observations Millikan showed that electric charge appeared only in integer multiples of a fundamental unit, e , whose modern value is approximately 1.602×10^{-19} coulomb.

Every elementary particle discovered so far has a definite charge ne where n is an integer, usually $+1$, 0 or -1 . Many particles have been discovered in the past 25 years, most of them belonging to the class known as hadrons which experience the strong nuclear force. The study of hadrons revealed an order and symmetry which was interpreted by M. Gell-Mann and by G. Zweig as being caused by a substructure of constituents, named

quarks, having fractional charges of values $-\frac{1}{3}e$ and $+\frac{2}{3}e$. Antiquarks would have opposite-sign charges to these. Various quark models, including some which do not involve fractional charge, have had considerable success in explaining features of high energy physics, and the quark hypothesis is generally accepted today. Many searches for free quarks have been performed, but apart from an occasional false alarm have all proved negative. Indeed there are theories in which interquark forces increase with distance and quarks therefore are necessarily confined within hadrons. A comprehensive review of the search for quarks was written in 1977 by L. W. Jones (*Rev. Mod. Phys.* **49**, 717; 1977).

Millikan's droplets had masses of around 10^{-14} kg and the absence of fractional charge on around 100 droplets gives an upper limit of about 10^{-15} for the number of free quarks per nucleon. It is possible to obtain a significant improvement in sensitivity by using much more massive objects, but then a stronger force is needed to balance them against gravity, and magnetic levitation can be used. In 1977, Fairbank and his colleagues published results from experiments which made use of the magnetic repulsion experienced by spheres of superconducting material. In a cryogenic apparatus, niobium spheres of mass 9×10^{-8} kg were levitated in a shaped magnetic field in which they had a natural vertical oscillation period of 0.8 Hz. An alternative vertical electric field of the same frequency was applied. The amplitudes of oscillations were measured and from these the net charges on the spheres were determined. The charge could be changed by $\pm e$ by bombarding the sphere with positrons or electrons from radioactive sources, so that as in the Millikan experiment, a series of charge values, differing by unit steps was obtained for each sphere. The experiment was performed with eight spheres, six of which showed zero residual charge. However sphere No 6 gave a residual charge of $+\frac{1}{3}e$ (to an accuracy of a few percent) on the two

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occasions on which it was measured. Sphere No 3 gave a residual charge of $-\frac{1}{3}e$ on its first measurement, but after removal from the apparatus, cleaning in organic solvents and reinsertion gave zero charge. The authors went to considerable effort to understand and eliminate possible spurious effects which could mimic fractional charges. The main background forces are caused by induced and permanent electric dipole moments and by trapped magnetic flux in the spheres. All were claimed to be too small to account for the results. The samples were all accurately spherical and had been prepared carefully before use. To improve their oscillations the spheres had been heated to high temperature before being used. Five were heated on a niobium sheet and three on tungsten. Both fractional charges were found on the latter spheres and the authors suggest that fractional charges had transferred from tungsten to niobium.

In the same month in 1977, G. Galilano, M. Marinelli and G. Morpurgo, from the University of Genoa, published results from their magnetic levitation experiment (*Phys. Rev. Lett.* **38**, 1255; 1977). Like Fairbank, Morpurgo and his colleagues had been investigating levitometer techniques for quark searches ever since Gell-Mann's original hypothesis, and indeed had been the first to publish the principles of the method. In their latest experiment, they used small iron cylinders, of mass 2×10^{-7} kg as their samples. The iron is attracted from above by an electromagnet, and a dynamic equilibrium against gravity requires an electronic feedback to the magnet current. However the method has the advantage of operating at room temperature. Vertical electrical plates were used and the cylinders were oscillated horizontally by an alternating potential difference. Again the amplitude is a measure of the residual charge on the samples. Five samples were investigated and no fractional charges were found. The total mass of material in the Stanford and Genoa experiments was quite comparable, about 1 mg or nearly 10^{21} nucleons each. Of course if the Genoa group had also obtained evidence for fractional charges of the required value, the case for free quarks would have been strengthened greatly. Their negative result, however, was not incompatible with the Stanford claims, both on statistical grounds, and because different materials were used. It is conceivable that quarks may have greater affinity to heavier nuclei, in which case niobium (nuclear charge $Z=41$) would be better than iron ($Z=26$), and tungsten ($Z=74$) would be better still. Nevertheless it was clearly possible that one of these difficult experiments was wrong, and in the exchange of

several letters in *Physics Today* during 1977 and 1978, Morpurgo and Fairbank each pointed out the advantages of his own method.

In addition, during 1977 R. Bland, D. Bocobo, M. Eubank and J. Royer at San Francisco State University (*Phys. Rev. Lett.* **39**, 369; 1977) examined tungsten directly in a Millikan apparatus using particles formed in an arc discharge between tungsten electrodes. Good measurements were taken on 69 particles. All were found to have zero residual charge to within a standard deviation of just over $0.01e$. However the total tungsten mass was only 3×10^{13} kg or about 2×10^{14} nucleons, and we can only conclude that tungsten is not several million times richer in free quarks than are niobium or iron.

The recent results published by the Stanford group, used an improved version of their apparatus. All three components of possible permanent electric dipoles could now be measured explicitly by moving a relatively large grounded copper sphere to various positions around 1 cm from the niobium sphere, and applying the alternating electric field symmetrically with respect to ground. The possibility of electric field gradients was further reduced by using optically flat and parallel quartz plates. Sphere No 6 which had charge $+\frac{1}{3}e$ in the previous experiment was used again and measured four times with an electric discharge occurring between levitations. Zero residual charges were found on the first, third and fourth measurements and $+\frac{1}{3}e$ on the second measurement. Sphere No 7 which had zero charge previously was used as a calibration during the same cooldown. During a second cooldown in the new experiment, sphere No 7 was used again, together with a new sphere, No 9. This latest sphere showed a charge of $+\frac{1}{3}e$ on each of the two occasions on which its residual charge was measured.

It is difficult to reach a satisfactory verdict on these experiments, and it is likely that most physicists will reserve judgment pending further results. It seems strange that the quarks in the Stanford experiment, if real, should behave in such a volatile manner. On the other hand, if the effect is spurious it is remarkable that the fractional values reported are just what are required for quarks. The experiments are obviously very difficult, but it is not clear from the published letters why so few measurements have been made. It would be useful to have more measurements, and preferably on samples of different sizes in the Stanford apparatus, and in addition, to suspend niobium samples on the iron in the Genoa experiment. □

Fluctuations in abundance of tropical insects

from Robert M. May

ONE tenet of earlier ecological speculation, still to be found in many popular pamphlets about 'ecology' and the environment, is the notion that complex and diverse communities (with many species and a rich web of interconnections among them) are more stable (with individual populations fluctuating less). A corollary is that, in the biologically crowded and species-rich tropics, populations should be more stable. While it is true that many temperate zone animals, particularly insects, do exhibit large population fluctuations, there is not much analogous information about tropical populations. Thus these theoretical ideas have received little in the way of empirical testing.

Recent work has considerably advanced our theoretical understanding of the possible range of relations between diversity and population stability in ecosystems (see for example *Nature* **276**, 117; 1978), undercutting any simple notion that complexity, of itself, promotes stability. But the empirical facts have tended to remain largely anecdotal. In Sierra Leone, Owen and Chanter (*J. Entomol.* **46A**, 135; 1972) found that a few species of *Charaxes* butterflies had fairly constant abundance, but these authors were concerned to emphasise their experience that most populations of tropical insects fluctuated a lot. In tropical America, Smith (*Carib. J. Sci.* **12**, 45; 1972) has shown a moth, *Urania fulgens*, to exhibit dramatic population explosions. Bigger (*Nature* **259**, 207; 1976) has reviewed data on some tropical crop pests, suggesting they fluctuate as much as temperate ones. Pursuing "arguments and data to show that tropical populations normally fluctuate no less violently than their appropriate counterparts of higher latitude", Leigh (in *Ecology and Evolution of Communities* Cody and Diamond (eds), Harvard University Press, 1975) showed that the amplitudes of fluctuation of some common vertebrate herbivores (ranging from northern temperate Isle Royale moose and Mt McKinley dall sheep to Serengeti buffalo and wildebeest) showed no consistent tropical-temperate differences. The difficulty is that most of these studies concern one, or at best a few, species, which can be argued to be exceptions.

In this context, a recent paper by

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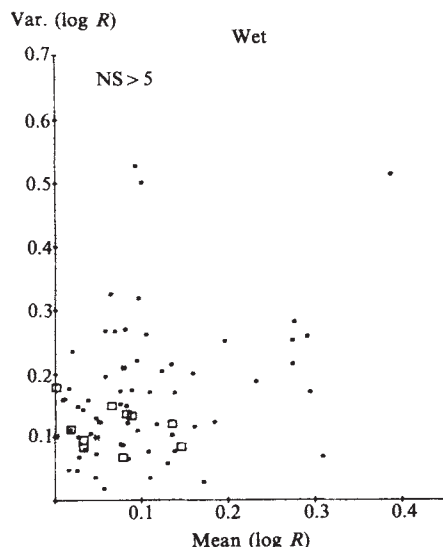


Fig. 1 The annual variability ($AV = \text{variance of } \log R$) plotted against the mean value of $\log R$, for various collections of data on insects. The open squares are for tropical insect communities, the solid squares for temperate ones, and the asterisks for (sub)arctic ones; in all cases, all populations are represented by at least five individuals ($NS > 5$).

Wolda (*Am. Nat.* **112**, 1017; 1978) does two important things. First, he presents data, collected from light traps, that constitute the best study yet made on the year-to-year fluctuations in abundance of tropical insects. Second, Wolda gives a systematic compilation and analysis of some 138 studies providing information about the average level of year-to-year fluctuation exhibited by groups of tropical and temperate insects.

For his own study, Wolda used three sets of light traps, located at sites in Panama. From the lowland tropical monsoon forest, on Barro Colorado Island in the Panama Canal Zone, he obtained 32,494 individuals that could be classified into Homopteran species (for a total of 565 species) from a ridge site, and 1,538 such individuals (222 species) from a valley site where the light traps were operated less often. The third site was on the edge of a town near Panama City, overlooking second-growth forest, and produced 53,680 classifiable Homopteran individuals (507 species). A few species from orders other than the Homoptera were also included in the overall tropical-temperate comparison, as were unpublished data for Blattellidae gathered by Fish. As discussed by Southwood (*Ecological Methods* (2nd ed), Chapman and Hall, 1978), collections from light traps contain biases that make them unreliable indicators of the total number of species in a region, or of the relative abundance of species. Light trap data should, however, pro-

vide a good estimate of the year-to-year fluctuations in abundance of any one given species; Wolda gives several lines of evidence (including independent estimates of abundance from netting) to suggest this is indeed the case for his study.

For each species, Wolda then calculates the ratio, R , that measures the change in abundance from one year to the next: $R = N_t / N_{t-1}$. For a group of species, one then obtains the frequency distribution of values of $\log R$, describing the year-to-year changes in abundance which occur within the group; the mean of this distribution measures the average population change for the group as a whole, and the variance measures the differences among the species. Note that Wolda's 'log R ' is the standard 'k-factor' of the pest control literature (see for example, Varley, Gradwell & Hassell *Insect Population Ecology*, Blackwell, 1973). Wolda christens the variance of the $\log R$ distribution the "annual variability", AV , and proceeds to use it to characterise the range of increases and decreases in species abundance within the community under consideration. High values of AV correspond to a wide range of values of $\log R$, with some species showing marked increase in abundance, and others marked decrease; small values of AV correspond to the set of species showing little variation in abundance. Wolda's own data are then analysed in detail, to show *inter alia* that statistical fluctuations arising from small sample sizes introduce relatively little bias if species represented in any one year by less than five individuals are excluded (provided there is information either about many species in any one year, or about a few species for several years).

Wolda condenses the results of his experiments into the AV versus the mean value of $\log R$ for the set of Homopteran species, and for a miscellaneous set of other species, at each of his three sites. These results are compared with those of a synoptic compilation of other work. The collections of species in these 130-odd other studies were variously obtained by baiting, beating, emergence traps, light traps, direct observation, pitfall traps, quadrat counting, sweeping, sticky traps, windowpane traps, and other forms of traps. They come from physical environments that may be roughly grouped as (sub)arctic, wet temperate, dry temperate, wet tropical, dry tropical, and a special group for obviously unstable situations (such as migrating moths, or insects invading a newly available area).

Figure 1 summarises the results of AV versus mean ($\log R$) for the sites

classified as 'wet': the open squares are for tropical insect collections; solid squares are temperate insects; asterisks are (sub)arctic. Discussing the pattern that emerges, Wolda says "the tropical data in [this figure] are in the same part of the graph as the majority and the best of the temperate data. [The data points based on 100 or more individual values of $\log R$ tend to have AV less than 0.2.] The temperate data scatter more, but there are many more of them. Both the smallest and the largest values of AV are from temperate insects. The conclusion can thus only be that the data suggest rather strongly that tropical insects have, on the average, the same annual variability as have insects from the temperate zone. There is no indication of a greater stability of tropical insects." Wolda sensibly does not subject the data in Fig. 1 to any statistical jiggery-pokery to buttress this assertion, and the reader is free to draw his own inferences from Fig. 1; I think Wolda's conclusion is reasonable.

For dry temperate, dry tropical, and 'unstable' collections of insect populations, there are fewer data points. These show more scatter (typically having higher values of AV and mean $\log R$) than the wet environments of Fig. 1, and the tropical data are clearly mingled amongst the temperate ones. As we might expect, the AV does show systematic increases in specific 'unstable' instances where the environment is disturbed or newly created. The AV for the community of insects and spiders that colonised the Lauwerszee polder, subsequent to its conversion to dry land in 1969, was 0.64, 0.49 and 0.37 for the years 1967–70, 1970–71 and 1971–72, respectively; this not only shows the predicted decrease as the community becomes more settled, but also is understandably larger than the corresponding AV of 0.10 for an older polder (Meijer *Oecologia* **16**, 185; 1974). For aquatic invertebrates upstream of an industrial outlet of cooling water in Britain, AV was 0.18; downstream it was 0.25 (Langford *Hydrobiologia* **47**, 91; 1975).

Given that tropical and temperate insect populations show similar levels of fluctuation in abundance under similar environmental conditions, Wolda goes further to claim that "tropical insects are not more to the K end of the r-K continuum of strategies . . . than their temperate counterparts". I think this goes too far. There are systematic trends in the life history strategies of many groups of animals, including insects, as one goes from the equator to the poles (see for example, Southwood *et al.*, *Amer. Nat.* **108**, 791; 1974). Such trends toward more K-ish behaviour among tropical

insects are not inconsistent with tropical insect communities exhibiting levels of population fluctuation comparable with temperate ones; the stabilising tendencies of such K-ish life history strategies can easily be offset by the lower dynamical stability that is characteristic of more complex (species-rich) ecosystems. This view that tropical and temperate communities may manifest similar levels of AV, accomplished by tropical populations exhibiting special features that help offset the intrinsically greater dynamical fragility of a complex system, is clearly a personal one, but it deserves to be considered.

In short, the body of information collected and compiled by Wolda suggests that tropical insect populations, including those from tropical forests, do not show systematically lower fluctuations in abundance than their temperate cousins. Insect populations from relatively dry areas, where the rainfall is low and unpredictable, do tend to fluctuate more; this tendency is, however, equally true for tropical and temperate insects. □

What's special about actin?

from Michael Geisow

THERE is little doubt that actin enables cells literally to shape up to their role in an organised community. Even loners like the amoeba do not dispense with this ubiquitous protein. Although fibrous (F)-actin is most familiar as the protein component of the thin filaments of skeletal muscle, current interests centre on non-muscle actins which are probably present in the cytoplasm of all cells. In non-muscle cells, F-actin forms the microfilaments frequently observed in association with the plasma membrane. Representative structures are the fibrous bundles within cell microvilli and stress fibres apparent in fibroblasts spread on a solid surface. These structures are frequently labile—the major distinction between muscle and non-muscle cells is that the latter have mechanisms for maintaining actin in its non-polymerised state (G-actin). These general observations raise three key questions. In non-muscle cells, what regulates the intracellular distribution of actin filaments and the degree of actin polymerisation? What determinants govern the attachment of actin to the plasma

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Third age of quantum chemistry

from Graham Richards

THE earliest quantum mechanical calculations of molecular properties were very crude and hoped only for agreement with experiment within an order of magnitude. The interest in this pioneering work arose from the fact that approximate agreement did provide some insight as to the reasons for the values of measured properties.

A second age depended on the advent of increasingly powerful computers. *Ab initio* calculations of molecular orbital wave functions boomed and continue to fill the scientific literature in many areas. These more accurate calculations are in much closer agreement with experimentally measured parameters. The complexity of the calculation often precludes the use of simple pictorial explanations and the answers cannot be preferred to experiment. Apart from proving the methods, this type of work receives most justification when applied to systems where the experiment is not a viable alternative to computation; perhaps the molecule is unstable or cannot be synthesised.

Now steady improvements in theoretical calculations have led to the dawning of a third age: calculations which are more accurate than experiment or at very least sufficiently accurate to be indispensable for interpretation. An example of such a case is the elegant spectroscopic experiment of Hunziker and his colleagues at the IBM Research Laboratory San Jose (Wendt, Hippler & Hunziker *J. chem. Phys.* **70** in the press).

The experiment concerns the near-infrared electronic absorption spectrum of the *cis* isomer of electronically excited triplet acetylene, formed by mercury-photosensitised reactions of acetylene, ketene and diazomethane. Although aliphatic unsaturated hydrocarbons in their lowest triplet electronic states are important chemical intermediates they have not been studied spectroscopically, in contrast to the widely investigated aromatic triplets. In its lowest triplet excited state acetylene adopts a planar bent

geometry with both hydrogens on the same side of the carbon-carbon bond (*cis*).

The key to identifying the spectrum was the theoretical work of Wetmore and Schaefer (*J. chem. Phys.* **69**, 1678; 1978) which showed that the observed features correspond closely with the predictions for the $^3A_2 - ^3B_2$ transition of acetylene. The spectroscopists were able to observe rotational and vibronic structure as well as deuterium isotope effects, again agreeing to a remarkable degree with the *ab initio* theoretical predictions.

An electric dipole transition between the triplet states is allowed for *cis* geometry ($^3A_2 - ^3B_2$) but parity forbidden in the *trans* case ($^3A_u - ^3B_u$) and the two forms are separated by a potential barrier of the order of 1 eV. The spectrum was observed using a photochemical modulation apparatus. With this technique small transient absorptions are observed by phase sensitive detection and reaction rates determined using phase shift measurements. These showed that the lifetime of the transient at 1.35 μm in the acetylene experiments was about 3×10^{-5} s. The principal feature of the spectrum is a single band at 1.35 μm supporting both the calculations and spectroscopic analysis which show that the geometries of the two electronic states are very similar. There is for instance a change in C-C-H bond angle of about 2° .

The formation of the excited *cis* acetylene is particularly efficient using Hg-photosensitised ketene and diazomethane. The suggested mechanism for this process involves the triplet state of methylene, CH_2 , in the reaction

The work represents perhaps a near perfect instance of theory being in harmony with experiment, each aspect vital to the other and the combination much more than the sum of the separate parts.

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membrane and to itself to form microfilament bundles? And last, but most crucial, how are the assembled actin fibres used to generate the diverse types of motion (or tension) with which they are apparently associated?

Very often the most profitable lines of work in complex biological systems have tackled the molecular character of the protein responsible. It is therefore surprising that although many actins

are readily purified in quantity, structural information is sparse. The few amino acid sequences which are known indicate that actin is very highly conserved; there are only 17 changes out of 375 amino acids between mammalian cytoplasmic actin and that isolated from the lower eukaryote *Physarum polycephalum* (Vanderkerckhove & Weber *Nature* **276**, 720; 1978). Five of these differences occur at the

amino terminus, which is highly variable in all actins.

Within this framework of close sequence homology, the non-muscle proteins (β and γ -isoactins) differ significantly from muscle proteins (α -actins). Non-muscle actins more closely resemble their counterparts in other species than muscle actins in their own species. There are 25 sequence differences between mammalian muscle and *Physarum* actin. Consistencies in the pattern of amino acid substitutions between the muscle and non-muscle classes of actin suggest that some of the differences reflect functional divergence rather than evolutionary 'drift'. For example, brain, platelet, *Acanthamoeba* and *Physarum* actins all have threonine (moderately polar) at position 129, while heart and skeletal muscle actins substitute valine (hydrophobic).

Slow evolution of amino acid sequence generally implies that a greater-than-average proportion of the polypeptide chain is determinant of biological function. For instance, in the cytochromes *c*, most of the numerous invariant residues are responsible for correct binding of the large, buried haem moiety. Sequence changes between species variants or even class variants of a given protein are usually accommodated at the molecular surface. Between the various actins, however, substitutions of charged (surface) amino acids are rare. In consequence, Vanderkerckhove and Weber point out that evolution of actin surface topography must be subject to unusually rigid constraints. In fact direct experimental support for this contention is provided by the weak immunogenicity of actin and the universal species specificity of some anti-actins.

Conservation of the surface structure of actin makes sense in terms of known functions of the protein. It can form extensive linear and lateral associations to produce F-actin and micro-filament bundles. In non-muscle cells these associations are readily reversible, but they are comparatively stable in muscle cells. Knight and Offer (*Biochem. J.* **175**, 1023; 1978) describe a method of chemically cross-linking favourably-disposed cysteine and lysine residues in adjacent monomers of muscle F-actin. This ingenious modification not only offers the opportunity of identifying regions of the actin monomer involved in polymer formation, but may also allow stabilisation of F-actin under a variety of conditions in order to study interaction with other molecules.

Both G and F-actins also associate with unrelated proteins. It has been known for some time that G-actin tightly binds pancreatic DNase I with $K_{app} = 5 \times 10^8 \text{ M}^{-1}$ (Lazarides & Lind-

berg *Proc. natn. Acad. Sci. U.S.A.* **71**, 4742; 1974). Rohr and Mannherz (*Eur. J. Biochem.* **89**, 151; 1978) report the natural occurrence of this complex in rat pancreatic secretion. The same workers also demonstrate that 5'-nucleotidase (present in rat bile) acts in the formerly intractable G-actin-nuclease complex to produce filamentous actin with the release of active DNase I (*FEBS Lett.* **95**, 284; 1978). The interaction between DNase I and actin led to the recognition that G-actin in spleen cells was bound to 'profilin'—a 16,000 molecular weight polypeptide. This complex was identical with a previously reported DNase I inhibitor from spleen. Because the profilin-actin interaction prevented the usual salt-induced polymerisation, this non-muscle actin could be crystallised as a 1:1 complex with profilin under conventional conditions of high ionic strength (Carlsson *et al. J. molec. Biol.* **105**, 353; 1976). Similarly, the prospect of 3-D structural analysis of muscle actin was made more likely by the introduction of a non-ionic polymer (PEG) as a means of crystallising G-actin (Oriol *et al. FEBS Lett.* **73**, 89; 1977). The reasons for the tight binding of G-actin to DNase I and profilin remain obscure, but may bear on the control of actin polymerisation which operates in non-muscle cells. G-actin also seems to form a polymeric complex with the unique molecule spectrin, a peripheral protein of the cytoplasmic face of erythrocyte membranes. It is not yet certain what the association state of actin is, but evidence favours the monomeric G-form.

F-actin appears to be catholic in its interactions with other cell proteins. Binding to α -actin, myosin, tropomyosin and troponin is established beyond doubt and is further indicative of the degree to which actin's molecular surface must be conserved. In non-muscle cells current reports frequently point to the importance of F-actin as a structure responsive to the distribution of membrane proteins. For example both actin and tubulin were found to co-cap with mouse B lymphocyte membrane immunoglobulin (Gabbiani *et al. Nature* **269**, 697; 1977) and with membrane receptors cross-linked by concanavalin A (Toh & Hard *Nature* **269**, 695; 1977). Flanagan and Koch (*Nature* **273**, 278; 1978) demonstrate that the amount of IgG co-extracted with actin by a myosin-actin affinity technique was proportional to the extent of cross-linked membrane IgG. Murine mastocytoma (p815) cells spontaneously shed plasma membrane from microvilli. In this exfoliated membrane a strong association exists between the histocompatibility antigen H-2 and actin (Koch & Smith *Nature* **273**, 274; 1978). It is significant

that the H-2 antigen is known to be a transmembrane protein and that both IgG and H-2 continue to associate with actin in the presence of detergents. Analogously, 5'-nucleotidase, freed from its membrane sites by the detergent action of bile salts, can also interact with actin in the form of its nuclease complex.

The strong implications of all these studies is that the extent of membrane attachment and possibly the degree of assembly of F-actin as a cytoskeletal element is sensitive to the state of association of a variety of membrane proteins. The stability of preformed membrane protein-actin associations after solubilisation from the membrane suggests direct binding of actin to membrane proteins by a transmembrane event. However there is as yet no unambiguous demonstration of direct interaction of actin with a transmembrane protein and most authors admit the possibility of intermediate components. The three questions posed initially have not been solved, but complete answers to some of them cannot be far off. It would be timely if the first answer to reach the press was the crystal structure of the source of all the problems; G-actin. □

Polymer mixtures

from Paul Culvert

A RULE which is taught in basic polymer courses is that polymers do not dissolve in one another because the entropy of mixing of large molecules is essentially zero. A review by Sonja Krause in 1972 (*J. macromolec. Sci., C*, **7**, 251) listed several hundred pairs of polymers of which only about 10% were compatible and this is probably an overestimate. With the current stress on modifying existing synthetic polymers rather than manufacturing new ones, studies on 'polymer alloys' have become fashionable and compatible systems are of real interest.

The basic Flory-Huggins theory of polymer solution thermodynamics, extended by Flory to include free volume effects, has been applied to polymer mixtures by McMaster of Union Carbide and now more simply by Patterson and Robard of McGill (*Macromolecules* **6**, 760; 1973; **11**, 690; 1978). The essence of the argument is that the entropy of mixing in a solution is proportional to the number of molecules and so becomes very small for high molecular weight polymers whilst

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the free energy of interaction between chains depends only on the volume of the system and so dominates at high molecular weights. Free volume differences give rise to small increases in free energy when similar liquids are mixed, so mixtures of similar polymers, for instance two polyolefins, will be incompatible. In order for a homogeneous solution to form there must be some preferential bonding between the unlike chains, such as hydrogen bonding or dipole interactions. This is characterised by a negative interaction parameter χ . Unfortunately this does not help a great deal to explain why some polymers are compatible. Although it seems plausible that some specific 1:1 dipole interactions could take place between polyvinyl chloride (PVC) and polyesters such as polymethyl methacrylate (PMMA) or poly(ϵ -caprolactone), it is hard to see what interaction makes poly(2,6-dimethyl 1-4-phenylene oxide) (PPO) compatible with polystyrene (PS). Such things can always be explained but not in a way that has any predictive value.

The experimental basis for compatibility is also rather weak. Early studies were done on the basis that cloudy samples were phase-separated whereas transparent ones were compatible. In fact transparency may just represent fine scale phase-separation or a refractive index match between the phases. Electron microscopy of stained samples can be used but this is limited to a scale of 100 Å or more and depends on having a good stain. The other approach is to observe the glass transition temperature(s) of the sample. Two transitions close to those of the pure polymers implies separation whereas one intermediate transition is taken to show compatibility. However this only works if the two starting polymers have transitions 100 °C or more apart and is misleading if phase separation or dissolution takes place during heating. There is also considerable doubt as to whether two transitions will be seen if separation is on a fine scale. Thus we can demonstrate phase separation but cannot demonstrate compatibility. It is also unclear whether complete dissolution can take place or if all mixtures contain at least local heterogeneity.

Kwei, Wang and Nishi of Bell Telephone Laboratories have looked at polystyrene-poly vinyl methyl ether mixtures by microscopy, vapour sorption and diffusion, and NMR (*Macromolecules* **7**, 667; 1974; **8**, 227; 1975). They were able to outline a phase diagram with a lower critical temperature above which separation occurred and found a suitably negative χ factor at 30 °C although χ was dependent on the mixture composition which is rather unsatisfactory. These bulk

measurements suggest homogeneous mixing but two T_g values were observed by wide-line NMR implying that two types of environment are present and hence heterogeneity. One of these polymers performed the interesting trick of going from cloudy to transparent to cloudy again as it was heated to 150 °C.

The polystyrene-polyphenylene oxide system is of technical interest since PPO is usually modified by addition of PS which reduces the melt viscosity so that the polymer can be processed. Workers at General Electric and at University of Massachusetts have investigated mixtures of PPO with styrene-*p* chlorostyrene copolymers (*Macromolecules* **7**, 902; 1974; **11**, 151; 1978). Phase separation occurs when the copolymers contain less than 33% styrene. A single glass transition was seen in the compatible systems but this broadens significantly close to the critical composition, dielectric loss peaks broaden similarly, suggesting that there are large local composition variations before phase separation occurs.

Another interesting system is poly vinylidene fluoride (PVF2)-polymethyl methacrylate which has been studied at Bell Telephone Laboratories and at Groningen (*Polymer* **19**, 173; 1978; *Macromolecules* **9**, 603; 1976; **11**, 766; 1978). PVF2 crystallises out of the

homogeneous mixture so that analysis of its melting temperature allows a χ parameter to be determined. It is small and negative as expected. PVF2 is compatible with isotactic, syndiotactic and atactic PMMA whilst PVC is incompatible with isotactic PMMA but miscible with the other two up to a 1:1 ratio of PVC to PMMA monomer units (*Polymer* **16**, 201; 1975). This indicates that some specific chain pairing is occurring which depends on the conformation of the PMMA and that these pairs are miscible with PVC but separate in excess PMMA.

Thus there is evidence in compatible systems for local segregation of each polymer and/or pairing of unlike chains. The structure of these materials must have much in common with that of block and graft copolymers where each chain is itself a mixture of two or more polymers. The 1977 ACS Symposium on Polymer Alloys (eds Klempner & Frisch, Plenum, 1977) shows the extent of industrial interest in these systems. As well as the familiar use of grafted elastomer phases to toughen brittle plastics it should be possible to improve solvent resistance, fire retardancy and oxidation resistance. However perhaps the most interesting possibility is the development of two phase or compatible polymers for selectively permeable membranes. □

Complexities of vitamin D metabolism still increasing

from Alastair Hay

THERE is no disputing the activity of the kidney metabolite of vitamin D₃, 1,25(OH)₂D₃. It is an active hormonal form of the vitamin essential for calcium and phosphorus absorption from the gut, and mobilisation of calcium from bone. There is dispute, however, as to whether it is the only hormone of vitamin D. The second contender for hormonal status is the other kidney metabolite 24,25(OH)₂D₃. It has been suggested (Kanis *et al. Brit. Med. J.* **1**, 1382; 1978) that this metabolite may also be a calcium-regulating hormone in man.

The arguments over the significance of these metabolites for calcium regulation were two of the themes considered at a recent workshop* on vitamin D.

To reinforce the point that 1,25(OH)₂D₃ is important, values for this metabolite were quoted for an array of clinical conditions. In contrast, 24,25(OH)₂D₃ measurements were less numerous but perhaps more contro-

versial. Serum measurements of 1,25(OH)₂D₃ were reported by B. Lund (Frederiksberg Hospital, Denmark) to be two to three times higher than normal in patients with hyperparathyroidism and acromegaly, and to be very high (~200 pg ml⁻¹) in children under 2 years of age. Values for 1,25(OH)₂D₃ in children fell progressively with increasing age but rose again during the adolescent growth spurt. Acromegaly is a syndrome in which there is an overproduction of growth hormone. This, together with the observations made in children, suggested to Lund that growth hormone is indeed involved in the regulation of 1,25(OH)₂D₃ production.

Lund reported low serum measurements of the metabolite in patients with senile osteoporosis and femoral neck fractures. Similarly in subjects with hypoparathyroidism he observed serum values of 20 pg ml⁻¹ compared with the 30–35 pg ml⁻¹ of control subjects. Serum values of 20 pg ml⁻¹ were also noted in subjects with kidney transplants. M. R. Haussler (University of Arizona Medi-

*The 4th Workshop on Vitamin D was held in Berlin on 18–22 February, 1979.

cal College) also reported some low serum $1,25(\text{OH})_2\text{D}_3$ values. He measured 10 pg ml^{-1} in subjects with D-dependent rickets but in D-resistant rickets the measurements were 32 pg ml^{-1} and were similar to control subjects—a surprising finding, for the low serum phosphate levels in these subjects might have been expected to produce higher $1,25(\text{OH})_2\text{D}_3$ values. Nevertheless, Haussler reported that treatment with $1,25(\text{OH})_2\text{D}_3$ cured the rachitic lesions of D-resistant rickets and was similarly effective in the treatment of a patient with tumour-induced osteomalacia with a serum $1,25(\text{OH})_2\text{D}_3$ of $17\text{--}18 \text{ pg ml}^{-1}$.

One of the more curious twists to the $1,25(\text{OH})_2\text{D}_3$ story is Haussler's observation that female spawning trout have levels of the metabolite in excess of 75 pg ml^{-1} . The metabolite is said to be important for phosphate regulation in the eel (McIntyre *et al.* *J. clin. Endocr.* 5(Supp.) 85S; 1976) yet neither D. R. Fraser (see *News & Views* 273, 426; 1978) nor M. R. Holick (Massachusetts General Hospital) has been able to detect this or other dihydroxy metabolites of vitamin D_3 in fish receiving isotopically-labelled vitamin. In view of the specificity of Haussler's assay many would assume that it is indeed $1,25(\text{OH})_2\text{D}_3$ he is measuring. However, the observations of Fraser and Holick suggest that it may not be this metabolite, but perhaps one of a closely related structure.

Several authors stressed that $24,25(\text{OH})_2\text{D}_3$ may be important for calcium action in bone but not everyone is convinced that it is. S. Edelstein (Weizmann Institute, Israel) presented evidence that $24,25(\text{OH})_2\text{D}_3$ seems to be necessary for endochondral ossification of cartilage and bone. J. Kanis (University of Oxford) says the metabolite increases calcium retention in man and that this may be associated with increases in skeletal calcium accretion. And H. M. Malluche (University of Southern California School of Medicine) reported that in chicks neither $1,25(\text{OH})_2\text{D}_3$ nor $24,25(\text{OH})_2\text{D}_3$ would maintain bone integrity on their own, but that both metabolites were required for this purpose, a view put forward earlier by Ornoy *et al.* (*Nature* 276, 517; 1978). S. W. Stanbury (University of Manchester) questions whether $24,25(\text{OH})_2\text{D}_3$ is so important for calcification of bone. In subjects treated for vitamin D deficiency with 1,000 IU of $^3\text{H-D}_3$ Stanbury observed $1,25(\text{OH})_2\text{D}_3$ and $25,26(\text{OH})_2\text{D}_3$ in the serum in the first few days but no $24,25(\text{OH})_2\text{D}_3$. In some cases $24,25(\text{OH})_2\text{D}_3$ was not seen until 6 or 10 days after the start of vitamin D therapy. In view of the fact

that changes in the calcification front of bone are seen 3–5 days after the administration of vitamin D, Stanbury queried whether $24,25(\text{OH})_2\text{D}_3$ was the principal metabolite responsible for mineralisation. Could it be that $25,26(\text{OH})_2\text{D}_3$ is a more important metabolite for mineralisation he asked. Stanbury also reported that serum levels of $25,26(\text{OH})_2\text{D}_3$ were $0.5\text{--}0.8 \text{ ng ml}^{-1}$ and about a third of the $24,25(\text{OH})_2\text{D}_3$ values in D-replete subjects. J. L. H. O'Riordan (Middlesex Hospital) on the other hand found much lower $25,26(\text{OH})_2\text{D}_3$ values ($178\text{--}336 \text{ pg ml}^{-1}$) using a radioimmunoassay. The antibody used by O'Riordan has been developed for use in measuring $1,25(\text{OH})_2\text{D}_3$, however, in view of its high, and as yet unexplained, cross-reactivity with the other dihydroxymetabolites of vitamin D_3 it is being used to measure $25,26(\text{OH})_2\text{D}_3$.

Measurements of $24,25(\text{OH})_2\text{D}_3$ still tend to be controversial chiefly because of the twofold difference reported for UK and US subjects. C. M. Taylor (University of Manchester) says that $24,25(\text{OH})_2\text{D}_3$ values are about 8% of the $25(\text{OH})\text{D}_3$ values whereas Haddad *et al.* (*Arch. Biochem. Biophys.* 182, 390; 1977) claim that in the US they are about 20%. Furthermore, Taylor says that there is no detectable $24,25(\text{OH})_2\text{D}_3$ in anephric subjects whereas Haddad, H. F. DeLuca (in *Proc. VII International Congress of Nephrology*, Montreal, 1978) and R. L. Horst (University of Wisconsin) say there is. However, it seems that these differences between the UK and US laboratories may not be irreconcilable. There are two possible explanations.

The first is one of three new metabolites of vitamin D_3 reported by H. F. DeLuca (University of Wisconsin) one of which comigrates with $24,25(\text{OH})_2\text{D}_3$ on Sephadex LH 20 chromatography, but which is separated from it on high performance liquid chromatography (HPLC). The metabolite is a lactone involving carbons 23 and 25 of the D_3 side chain. Although the lactone accounted for a sizeable portion of the $24,25(\text{OH})_2\text{D}_3$ peak on Sephadex when chick serum was used, Horst said that he had not seen it in human plasma. The other alternative is the $25,26$ -dihydroxymetabolite of vitamin D_2 which we (Hay & Jones *Clin. Chem.* 25, 473; 1979) have reported also to comigrate with $24,25(\text{OH})_2\text{D}_3$ on Sephadex LH 20. Taylor confirmed this observation, but reported that this comigration also occurs on HPLC. It seems, therefore, that where vitamin D_2 is consumed in appreciable quantities in the diet—as it is in the US—that $25,26(\text{OH})_2\text{D}_2$ may be assayed together with $24,25(\text{OH})_2\text{D}_3$. This D_2 metabolite may also account for the $24,25(\text{OH})_2\text{D}_3$ values recorded in anephric subjects in the US.

Measurements of both $25,26(\text{OH})_2\text{D}_2$ and $24,25(\text{OH})_2\text{D}_3$ will be required before this can be answered definitively. Perhaps when these results are available the UK and US results will be seen to agree and investigators can turn once more to elucidate the actual functions of the $24,25$ and $25,26$ dihydroxy metabolites of vitamin D, and indeed that of all the other metabolites discovered over the past year, for it is quite clear that many doubts exist about some of the theories put forward so far. □



A hundred years ago

A STRIKING and highly promising line of research has been recently adopted by Mr. Muybridge, of San Francisco, at the instance of Governor Stanford, viz., the instantaneous photography of animals in motion. Some of his earlier photographs of a fast-trotting horse presented attitudes wholly unexpected, and they were even thought absurd. The method latterly adopted seems to have been making the horse trot or gallop past twelve cameras, arranged in series, and by breaking threads stretched across its path, release an electric current, which effected exposure of the plates. Thus the flying steed was obtained in every position. Mr. Muybridge gave public exhibitions of what had been done. The small negatives were magnified into life size, and projected on a screen, so that every motion was visible. These exhibitions do not seem to have been appreciated by the San Franciscans. The *Scientific American*, however, and afterwards *La Nature*, have published cuts taken from the photographs, and much general interest has been awakened in these researches. Among those specially interested is Prof. Marey, who desired to be put in communication with Mr. Muybridge, as he wanted to ask his aid in solving certain physiological problems, so difficult to solve otherwise; e.g., questions connected with the flight of birds. He had been dreaming of a kind of photographic gun, to seize the bird in an attitude or series of attitudes of flight. What beautiful zootropes, too, might be had! Mr. Muybridge's cartoons representing the fast gallop give a key to the breaking down of so many horses. It appears as though one fore-leg had to sustain the whole weight of horse and rider while the body is moved along five feet. And just before the foot is raised, a perpendicular from it would strike the back of the saddle; so that there is immense leverage, the centre of gravity being thrown so far forward of its support, and the tendons must have a terrible tension. These inquiries are being further developed by the liberality of Governor Stanford and the skill of Mr. Muybridge, and valuable results may doubtless be looked for.

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articles

Ancient crustal metamorphism at low p_{H_2O} : charnockite formation at Kabbaldurga, south India

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Arrested charnockitic conversion of amphibolitic gneiss at Kabbaldurga, Karnataka State, south India, was studied mineralogically. Iron-rich pyroxenes were generated from amphibole in patches and stringers without melting. The dark colour of charnockite arises from numerous tiny veins of chlorite and manganese-bearing calcite, particularly in feldspars. The metamorphism was effected by very local, mainly grain-boundary, migration of volatiles low in H_2O , and probably dominantly CO_2 . This was followed by vein alteration at lower temperatures from volatiles richer in H_2O . The volatiles are ascribed to massive liberation from the mantle in upwelling areas, and this may have been an important process in the evolution of the deep continental crust.

CHARNOCKITE is a widespread orthopyroxene-bearing granitic rock in the Precambrian shield of southern India¹. It commonly has dark green to brown coloured fresh surfaces². Closely related intermediate to basic rocks at the Madras type locality were called the 'charnockite suite'². The abundant global distribution of the charnockite suite in Precambrian terrains is a key issue in discussions of crustal evolution³.

Charnockite apparently derives from both igneous and sedimentary rocks⁴⁻⁸ by a particular kind of metamorphism. The associated pore fluids must have been low in H_2O because the association orthopyroxene-potassium feldspar is limited to H_2O pressures below 700 bar, being replaced by either biotite-quartz or melting at higher H_2O pressure⁹. Carbon dioxide is a probable diluent: primary CO_2 -rich inclusions are characteristic of granulites¹⁰ and charnockites¹¹. The present mineralogical study of arrested charnockite development at a southern India locality shows that conversion of amphibolitic gneiss to charnockite was effected without melting by grain-boundary migration of low p_{H_2O} volatiles, presumably mainly CO_2 . A copious, possibly primitive, source of volatiles, such as a mantle plume or diapir, must be invoked.

Partial conversion of Archaean (~2,700 Myr) amphibolitic gneiss to charnockite at a quarry in Kabbaldurga, Karnataka^{12,13}, presents the opportunity for microanalytical study of amphibolite to granulite transition in both acid gneisses and basic enclaves.

The Kabbaldurga and similar occurrences in southern Karnataka¹⁴ may be the northernmost 'front' of charnockite

development which culminates in the great massifs of the Biligirirangan and Nilgiri hills. All features of the cratonic portion of Karnataka, including the Dharwar schists and younger granites, as well as the dominant Peninsular gneiss, can be traced southward as relics into the charnockite massifs.

Such large-scale conversion to granulite would culminate in a stable, refractory, and inert continental mass low in the incompatible and heat-producing elements¹⁵.

Mineralogical changes in charnockitisation at Kabbaldurga

The altered gneiss has the characteristic dark colour of charnockite and contains hypersthene in the most reconstituted specimens. Diffuse patches and stringers of charnockite, from a few centimetres to metres across, partially or completely obliterate the gneissic foliation and strongly suggest very local action of volatiles, whose access is controlled by shearing or other deformation. All stages of the amphibolite to granulite conversion occur in both the dominant acid gneiss country rock (Peninsular gneiss) and basic enclaves which are undoubtedly dykes of more than one generation, variously contorted and boudinaged. Rock analyses¹⁴ of unaltered gneiss and completely charnockitised gneiss (Table 1), show little change in bulk composition during the process. A slight depletion in K_2O may be suggested. That some basic dykes have conspicuous charnockite selvages might indicate metasomatic interchange between acid and basic rocks as a primary cause of the conversion, except that many dykes, although partially converted to granulite, have no such marginal alteration and some have it only on one side. Channelling of volatiles by less pervious basic horizons seems more probable.

Various degrees of conversion of amphibolitic to granulitic mineralogy are identifiable macroscopically and in thin section in both acid gneisses and their basic inclusions. A few specimens show some metasomatic changes related to charnockite development.

The least-altered acid gneisses are quartz-feldspar rocks with thin subsidiary mafic bands, mostly biotite-rich, but some with both green-brown hornblende and biotite. Magnetite and apatite are ubiquitous accessories. The potassium feldspar is deformed microcline with mottled and strained cross-hatched twinning and exsolution. Antiperthitic oligoclase shows strain-induced albite twinning. Undulatory quartz tends to be lenticular. Oligoclase-quartz myrmekite occasionally marks triple junctions of feldspars and quartz.

The first stages of alteration are signalled in hand-specimen by a dark greenish-brown discoloration of the larger, normally white, feldspar grains. In more advanced stages, the rock as a whole assumes a dark mottling in feldspar-rich portions with some interruption of gneissic banding. In thin section, the minerals are normal except for enhancement of strain effects. Feldspar grains are conspicuously laced with very thin greenish veinlets, which sometimes cut across grains but as often follow grain boundaries. Little change in mineralogy is evident.

At extreme alteration, the hand-specimen is typical dark-brown charnockite. All gneissic texture is gone, and hornblende and biotites are smaller and fewer. Orthopyroxene appears abruptly in large bent grains whose margins are indeterminate because of late alteration to a dark-red, fine-grained material. Therefore, no clear-cut evidence of the relationship of orthopyroxene to biotite and hornblende exists, although these minerals presumably were its precursors. The orthopyroxene commonly has fine exsolution lamellae and about 10° of inclined extinction and is pleochroic in pale pink and green. Orthopyroxene is most abundant in mafic bands associated with large magnetite grains with quartz inclusions and intergrown ilmenite, but occasional large isolated grains are surrounded by quartz and potassium feldspar.

The thin greenish veinlets are more abundant in the most charnockitic specimens. Many feldspar grains are laced with them, and some cut across quartz grains. Many grains are enveloped by veinlets (Fig. 1), which occasionally open to tiny pods of birefringent material. The veinlets are accompanied by innumerable very brightly birefringent specks.

The dark colour of Madras charnockites was attributed¹⁶ to many apparently similar greenish veinlets cutting and enveloping feldspars. Several hours' contact with warm HCl bleached the charnockite to the appearance of a granite, undoubtedly due to removal of vein material. Chlorite was suggested as the vein filler¹⁶, and some iron loss has been observed after leaching. Qualitative microanalysis of vein material has shown major iron content¹⁷.

The basic inclusions show a more gradual progression to granulite mineralogy. In little-altered specimens the assemblage is green-brown hornblende, oligoclase or andesine and quartz, with smaller amounts of biotite, some of which is probably retrogressive. Diopside first develops in fine-grained symplectites with plagioclase marginal to large hornblende grains.

Hornblende relics in symplectite patches are optically continuous. Various stages of symplectite coarsening culminate in equigranular diopside-hornblende-plagioclase rocks. Veining of plagioclase is common, and large hornblende crystals are frequently cut by coarse biotite-calcite veins.

A few samples show strong metasomatic interaction. Gneissic contacts with granulitised metabasites may be converted to plagioclase-hornblende-diopside-quartz rocks with interstitial potassium-feldspar and typical dark charnockitic appearance. A biotite-rich altered dyke-rock has abundant large potassium-feldspar porphyroblasts associated with diopside. No hornblende is present. This dyke of ~1 m thickness has a 1 m-wide charnockite selvage on one side only. The metamorphic fluids were apparently able to transport potassium.

Microanalytic methods and results

Minerals were analysed with a ARL electron microprobe with a solid-state energy-dispersive system. Beam spot-size was 1 µm and beam current was 60 nA at 15 kV accelerating voltage.

The mafic minerals (Table 1) are richer in iron than those of the Madras type locality^{4-8,18}. The Fe/Mg ratio is higher for the minerals of the gneisses than for the basic rocks. The orthopyroxene (Fs₅₅) has a greater Fe/Mg ratio than any of the other mafic minerals, as was found for the Madras charnockites¹⁸. Note the high CaO and MnO contents of orthopyroxene. Biotite and hornblende become slightly but definitely more magnesian when orthopyroxene occurs in the acid rocks. No such change is evident as diopside appears in the basic rocks. Symplectitic diopside is not different from coarser, recrystallised diopside. The plagioclases do not change from An 27-35 as pyroxene develops in the acid rocks. Primary plagioclase in the basic rocks has a similar range with some slight normal zoning of large crystals. The symplectitic plagioclase in As 33 (Table 1) is An 24, compared to An 31 for the coarse primary plagioclase. Other than the slight zoning of some plagioclase, no significant zoning of minerals was found, thereby indicating thorough attainment of local equilibrium.

The thin greenish veins were scrutinised closely. The traces of extremely thin veins on polished feldspar surfaces gave significant iron and magnesium analyses, although the dominant contributor was wall-rock feldspar. Occasionally, a tiny speck of carbonate or chlorite in a vein gave practically uncontaminated

Table 1 Analyses of rocks and minerals from Kabbaldurga Quarry

	Whole rock		Biotite		Hornblende			Pyroxene		Plagioclase		Vein minerals		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
SiO ₂	69.44	70.64	37.7	36.9	43.5	40.8	44.6	51.0	51.8	62.6	64.0	61.2	32.9	—
Al ₂ O ₃	13.79	13.82	13.6	13.6	9.7	12.9	9.5	0.9	1.6	23.4	23.8	25.2	11.3	—
TiO ₂	0.59	0.53	4.0	4.2	1.8	1.5	0.8	0.1?	0.0	0.0	0.0	0.0	0.0	—
Fe ₂ O ₃	1.72	1.50	18.8	19.6	19.2	19.3	16.1	31.0	11.6	0.0	0.0	0.0	32.2	—
FeO	2.54	2.18												
MnO	0.02	0.07	0.0	0.0	0.3	0.5	0.1?	1.6	0.3	0.0	0.0	0.0	0.2	4.8
MgO	1.01	0.82	11.9	11.2	9.7	8.3	11.6	14.5	11.6	0.0	0.0	0.0	11.6	—
CaO	2.84	2.74	0.0	0.0	10.8	11.0	11.9	1.0	21.9	4.0	3.9	6.3	0.4	61.2
Na ₂ O	3.85	4.11	0.0	0.0	2.0	1.9	1.8	0.1?	0.4	9.4	9.8	8.1	0.0	—
K ₂ O	3.73	2.91	9.7	9.7	1.5	1.5	1.4	0.1?	0.0	0.2	0.2	0.2	0.0	—
P ₂ O ₅	0.24	0.20	—	—	—	—	—	—	—	—	—	—	—	—
H ₂ O+	0.28	0.34	—	—	—	—	—	—	—	—	—	—	—	—
	100.05	99.86	95.7	95.6	98.5	97.7	97.8	100.3	99.2	99.6	101.6	101.0	88.7	66.0

- (1) Acid gneiss, average of two analyses; Herdsman, analyst⁹.
- (2) Charnockitised acid gneiss, average of three analyses; Herdsman, analyst⁹.
- (3) Biotite in completely charnockitised acid gneiss N3.
- (4) Biotite in little-altered acid gneiss N1.
- (5) Hornblende in completely charnockitised acid gneiss N3.
- (6) Hornblende in little-altered acid gneiss N1.
- (7) Hornblende intergrown with diopside in altered mafic dyke AS 33.
- (8) Orthopyroxene in completely charnockitised acid gneiss N3.
- (9) Clinopyroxene in altered mafic dyke AS 33.
- (10) Oligoclase in completely charnockitised acid gneiss N3.
- (11) Oligoclase in little-altered acid gneiss N1.
- (12) Oligoclase in altered mafic dyke AS 33.
- (13) Chlorite pod in vein, completely charnockitised acid gneiss N3.
- (14) Carbonate grain in vein, completely charnockitised acid gneiss N3.

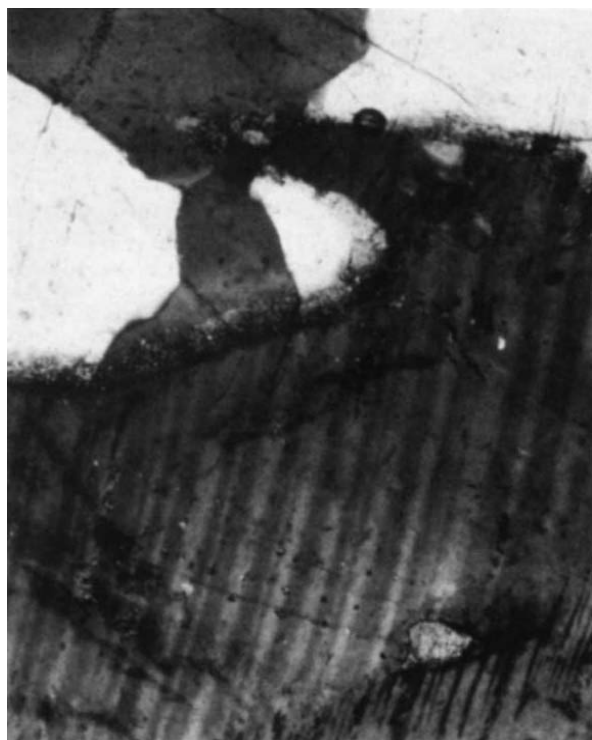


Fig. 1 Grain-margin veinlet, separating twinned plagioclase from quartz. Note tiny, brightly birefringent specks in veinlet, and bending of plagioclase twin lamellae. Most charnockitised gneiss (N3). Width of photo: 0.7 mm. Partially crossed polars.

analyses. Rare pods of chlorite in thicker veins gave good analyses. The Fe/Mg ratios of heavily contaminated analyses were the same as for the pod analyses, and the veins must contain the same kind of chlorite as the pods, plus calcite. The chlorite is an iron-rich, low-aluminium variety and the calcite is manganese-bearing (Table 1). Plagioclase immediately adjacent to a vein is moderately to highly albitised, but without recrystallisation. Potassium feldspar undergoes an antipathetic reduction in sodium within a few mm of a vein to a nearly sodium-free variety. Unusual mineral specks in the veins were rich in phosphorus and vanadium.

Conclusions and discussion

Several conclusions were drawn about the charnockite-forming event seen arrested in progress at Kabbaldurga.

(1) The granulite metamorphism involved localised action of volatiles low in H_2O and, by implication, rich in CO_2 . No concomitant large changes in temperature or pressure were required. The pyroxenes were generated from amphibole without any visible evidence of partial melting. Myrmekitic intergrowths at quartz and feldspar junctures are virtually devoid of K_2O and cannot be a partial melting product¹⁹. Compositions of co-existing mafic minerals are essentially unchanged when pyroxene appears, suggesting no major change of physical conditions. Slow migration of H_2O -poor volatiles could well have been controlled by shearing, or the locations of fold hinges or other deformational features, but strong temperature differences over spaces of a few metres could not have been produced by the migrating gases. Such action of reduced H_2O activity in metamorphic fluids at more or less constant temperature and pressure was invoked to explain the regional amphibolite to granulite transition in southern Norway²⁰. In the south Norway granulite terrain, as in many others, the primary fluid inclusions of minerals are dominantly CO_2 -rich and very low in H_2O (ref. 10).

(2) The event was at relatively high level. Unfortunately, mineral assemblages in the acid or basic rocks place few controls on temperature and pressure. However, absence of garnet gives

an upper limit of ~ 9 kbar for acid compositions if temperatures in the upper end of the amphibolite range, 600–700 °C, are appropriate²¹. The orthopyroxene is not rich enough in iron to be useful in orthopyroxene–olivine–quartz barometry²². The ratio of tetrahedral aluminium to total aluminium in hornblende is 0.79 in metabasite AS 33 and 0.81 in acid charnockite N3 (Table 1), compared with an average of 0.80 for hornblendes co-existing with biotites in the low-pressure granulites of the southern Yilgarn Block in Western Australia²³. Aluminium is low in pyroxenes. Three to five kbar may be appropriate for the Kabbaldurga granulites.

(3) The waning phases of the metamorphism were characterised by cooler solutions, richer in H_2O , confined to the same localised channelways. Chlorite and manganese-calcite in the veins indicate lower-grade mineralisation than the pyroxene-forming event. Qualitatively, the assemblage is lower-temperature than amphibole + epidote + vapour, and has an intermediate CO_2/H_2O ratio, bounded by ankerite and epidote for CO_2 greater than about 60% of the vapour²⁴.

The veinlets must be related intimately to the pyroxene-producing metamorphism because of the strict correspondence between abundance of veins and the appearance of pyroxene, both in acid and basic rocks. Unaltered gneisses a few yards away from heavily charnockitised gneisses do not have the veins. Even partially charnockitised gneisses in which feldspars are macroscopically discoloured have relatively few veins.

From these conclusions, the picture emerges of copious streaming of low- H_2O , presumably CO_2 -rich solutions, especially along pathways created by deformation. Water is purged and, with it, the minor incompatible elements normally resident in amphiboles, micas and epidote¹⁵. Temperatures may be elevated over broad areas, but gas-streaming is unlikely to raise temperatures significantly on a very local scale. Deeper portions of the crust are converted to refractory granulite. Perhaps there is a crustal level above which, for reasons of low temperatures and readily available H_2O , the process is not operative, and Kabbaldurga is a critical horizon. Such a picture accords with the depth–zone relationship of metamorphism proposed for southern Karnataka¹⁴, culminating in the extreme south with the deep-crustal charnockite massifs.

What is the origin of the postulated CO_2 -rich solutions? Calc-silicate rocks are locally abundant in association with charnockite terrains in the southern Indian shield, and may represent former carbonate rocks. Some may be linked to charnockite development, although it is difficult to see how occasional carbonate horizons could account for charnockite development on such a vast scale as the Nilgiri massif, for instance. No calc-silicate rocks crop out in the Kabbaldurga area. Graphite-bearing rocks are common in the Precambrian, but reaction of graphite with a pore water would produce a vapour which is at least 50% H_2O (ref. 25); amphiboles could not dehydrate in such a water-rich vapour without widespread melting. Primitive sources of CO_2 are increasingly invoked to convert large portions of the continental crust to granulite^{26,27}; ascending mantle convection 'plumes' may be the answer. If these were active in the Precambrian^{28,29}, a volatile phase of initially primitive CO_2/H_2O ratio, believed to be about 3:1³⁰, would be expelled by mantle dehydration and decarbonation. During invasion of the lower continental crust the H_2O component should cause widespread melting and be filtered out, leaving CO_2 -rich ascending solutions. If geothermal gradients were quite high, as expected over the plume, a P – T field of scapolite mineralisation may be avoided³¹ and the CO_2 could continue upward to drive out water from the overlying crust. During the cooling phase of the process, H_2O might again be released by crystallisation of granitic melt at deep levels. The H_2O might mix with CO_2 still coming off the mantle plume or liberated by decarbonation or scapolite conversion to zoisite. These more H_2O -rich solutions would then follow the earlier H_2O -poor solutions, quite plausibly along the same channelways, and could have deposited the chlorite–calcite vein assemblage. Alternatively, heavy CO_2 input might 'swamp' H_2O

resident in the crust during the height of the activity, but be insufficient to lower the H_2O fraction to less than about 40% of the total during the waning stages. Whatever the exact mechanisms controlling vapour composition, combined petrographical and geophysical field work, as, gravity or magnetic surveys, should be useful in implicating or exonerating a mantle diapir as a source of the vapours.

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1. Holland, T. H. *Mem. Geol. Surv. Ind.* **28**, 119 (1900).
2. Subramaniam, A. P. *Am. J. Sci.* **257**, 321 (1959).
3. Heier, K. S. in *The Early History of the Earth*, (ed. Windley, B. F.) 159–164 (Wiley, New York, 1976).
4. Howie, R. A. *Trans. R. Soc. Edin.* **62**, 725 (1955).
5. Cooray, P. G. *Am. J. Sci.* **267**, 969 (1969).
6. Rama Rao, B. *Bull. 18* (Mysore Geol. Dept, 1945).
7. Naidu, P. R. J. *Presidential Address, Ind. Sci. Congr.* (1964).
8. Sen, S. K. *J. geol. Soc. Ind.* **15**, 413 (1974).
9. Luth, W. C. *J. Petrol.* **8**, 372 (1967).
10. Touret, M. J. C. *r. hebdo. Acad. Sci. Séanc., Paris* **271**, Ser. D. 2228 (1970).
11. Ormaasen, D. E. *Lithos* **10**, 291 (1977).
12. Pichamuthu, C. S. *Nature* **188**, 135 (1960).
13. Ramiengar, A. S., Ramakrishnan, M. & Viswanatha, M. N. *J. geol. Soc. Ind.* **19**, 411 (1978).
14. Pichamuthu, C. S. *Indian Miner.* **6**, 119 (1965).
15. Heier, K. S. *Phil. Trans. R. Soc. A* **273**, 429 (1973).

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16. Howie, R. A. *Science Progr.* **52**, 628 (1964); *J. geol. Soc. Ind.* **8**, 1 (1967).
17. Oliver, R. L. & Schultz, P. K. *Min. Mag.* **36**, 1135 (1968).
18. Sen, S. K. & Sahu, J. R. *Contr. Miner. Petrol.* **27**, 239 (1970).
19. Amit, O. & Eyal, Y. *Contr. Miner. Petrol.* **59**, 95 (1976).
20. Touret, J. *Lithos* **4**, 239 (1971).
21. Green, D. H. & Lambert, I. B. *J. geophys. Res.* **70**, 5259 (1965).
22. Berg, J. H. *J. Petrol.* **18**, 33 (1977).
23. Stephenson, N. C. N. *Lithos* **10**, 9 (1977).
24. Harte, B. & Graham, C. M. *J. Petrol.* **16**, 347 (1975).
25. Eugster, H. P. & Skippen, G. B. *Researches in Geochemistry*, Vol. 2, (ed. Abelson, P. H.) 492–520 (Wiley, New York, 1967).
26. Tarney, J. & Windley, B. F. *J. geol. Soc. Lond.* **134**, 153 (1977).
27. Collerson, K. D. & Fryer, B. J. *Contr. Miner. Petrol.* **67**, 151 (1978).
28. Katz, M. B. *Precamb. Res.* **3**, 91 (1976).
29. Fyfe, W. S. *Chem. Geol.* **23**, 89 (1978).
30. Brey, J. & Green, D. H. *Contr. Miner. Petrol.* **61**, 141 (1977).
31. Goldsmith, J. R. & Newton, R. C. *Am. Miner.* **62**, 1063 (1977).

High magnesia liquids as the parental magma for ocean floor basalts

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The primary melt segregating from the upper mantle beneath an oceanic ridge is shown to contain ~18% MgO. Whereas these primary liquids are capable of generating the petrological features of the oceanic crust, this is not possible for tholeiitic basalts with 9–11% MgO which are highly fractionated. Compositions of liquids ranging from primary melts to high calcium picrites from the Tortuga ophiolite complex, Chile, are reported.

FOR several decades, the origin and diversity of basaltic rocks has been a foremost problem in igneous petrology. After Bowen¹ designated basalt as a primary magma which has risen to the Earth's surface chemically unmodified from its origin in the upper mantle, the predominant opinion among petrologists has been that basalts are primary mantle melts or have undergone only minor degrees of chemical modification during their ascent to the Earth's surface. In contrast to this hypothesis, O'Hara's^{2,3} review of pertinent phase equilibria in natural and synthetic basaltic systems led to the conclusion that basaltic magmas must be the result of advanced degrees of fractional crystallisation, especially at low pressures.

A major obstacle to reconciliation of these two opposing views on basalt genesis is that while phase equilibria indicate that basalts are highly fractionated, the overwhelming abundance of basalts stands in stark contrast to the complete absence, in the oceanic basins, of the proposed picritic magma of O'Hara. As a result, the concept of ocean floor tholeiitic basalt as a primary magma remains as a well accepted, albeit *ad hoc*, hypothesis.

The recent discovery of MgO-rich dykes representing liquid compositions in the Tortuga ophiolite complex, Chile, necessitates a re-evaluation of the nature of the genetic link between 'primitive' ocean floor tholeiites (9–11% MgO) and the upper

mantle. From several independent lines of reasoning, it is possible to demonstrate that magmas with ~18% MgO are primary mantle melts and the parental magmas for relatively evolved basalts with 9–11% MgO.

Composition of the oceanic crust

Ophiolites, as remnant sections of oceanic or marginal basin crust, can provide numerous constraints on the nature of magmatic evolution at a spreading ridge. A complete ophiolite sequence consists of a wide variety of igneous lithologies whose internal relationships have been described elsewhere^{4–6}.

From published data in the Vourinos ophiolite⁷, the Quebec ophiolites⁸, and the Newfoundland ophiolites⁹ as well as my unpublished data from the Tortuga ophiolite, Chile, a petrological columnar section of the oceanic crust has been constructed (Fig. 1). The thicknesses of the various petrological divisions have been constrained from geophysical data¹⁰ and measured sections in ophiolites^{7,8,11}.

An important chemical feature of the oceanic crust is that the crust becomes more enriched in ferromagnesian minerals, with increasing depth. The fractional crystallisation of ferromagnesian minerals from the magma results in the accumulation of olivine, pyroxene, plagioclase, and spinel in the magma chamber, with the extrusion of evolved basaltic liquids onto the floor of the ocean. This results in a mineralogical and chemical layering of the oceanic crust for which, excluding volumetrically minor trondhjemites and other silicic liquids, the most differentiated portion (with highest FeO/MgO) is the sheeted dykes and pillow lavas. Thus, even those sheeted dykes and pillow lavas with 9–11% MgO have undergone significant fractional crystallisation, as predicted by experimental work², and their compositions have been chemically modified since separation from the upper mantle.

Indirectly, the composition of the parental magma separating from the upper mantle can be estimated from Fig. 1 and analyses

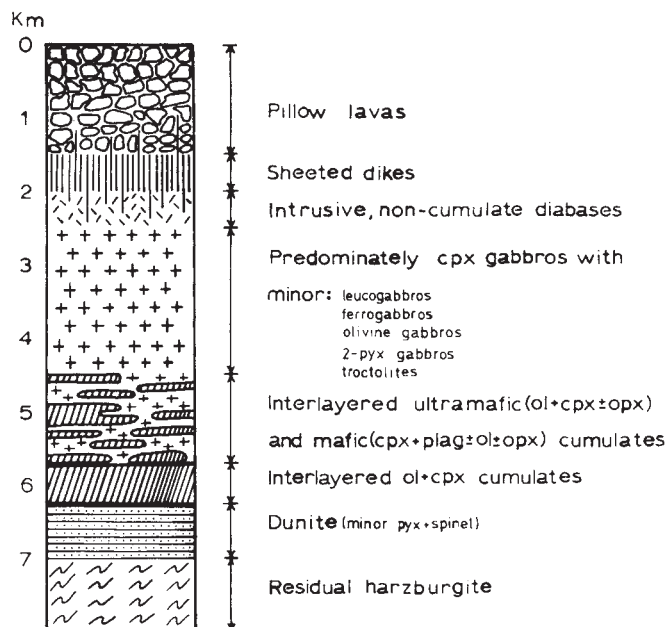


Fig. 1 A columnar section of the oceanic crust constructed from lithologies observed in ophiolites. The thicknesses of various lithological units have been constrained from measured sections in ophiolites and geophysical data. See text for details.

of rocks from the Tortuga ophiolite (Table 1). (The term parental rather than primary magma is used here to include the possibility of sub-Moho fractionation which may occur locally.) The bulk composition of the oceanic crust, used here to include all of the igneous rocks above the 'residual' harzburgite, must be equal to the composition of the parental magma as it enters the magma chamber before its extended history of fractional crystallisation and magma mixing, which result in the chemical and mineralogical layering observed in ophiolites (Fig. 1). It is critical while estimating the composition of the oceanic crust to include not only the fractionated magmas which erupt onto the ocean floor in the form of pillow lavas, but also the crystals which have accumulated within the magma chamber.

For these calculations, the oceanic crust can be divided into three sections: (1) pillow lavas, sheeted dykes, and intrusive diabases representing liquid compositions extracted from the upper portions of the magma chamber; (2) cumulate clinopyroxene-bearing gabbros with minor, associated leucogabbros, ferrogabbros, olivine gabbros, two-pyroxene gabbros, and troctolites; and (3) cumulate ultramafic and transition-zone lithologies. The composition of the average sheeted dyke in the Tortuga complex (D. E., unpublished data) is used as the representative composition for the pillow lava-sheeted dyke-intrusive diabase section of the oceanic crust. A comparison of the average sheeted dyke in Tortuga with the average Atlantic Ocean tholeiite glass¹² and the average FAMOUS glass¹³ demonstrate the chemical similarity of the Tortuga ophiolite with oceanic crust (see Table 1). As a model composition for the clinopyroxene-bearing gabbros (with the minor constituents described earlier) the average composition of the 'gabbros' from the Tortuga complex are used (D.E., unpublished data). These are genetically related to the dyke compositions and are chemically and mineralogically similar to gabbros from the ocean floor. Whereas chemical analyses for transition zone and cumulate ultramafic rocks are not available in sufficient quantity to reconstruct the chemical nature of this lowest region of cumulate rocks, the relative proportions of minerals can be estimated from Vourinos⁷ and Quebec⁸ with the mineral chemistry from these sources supplemented by the data of Church and Riccio⁹ and England and Davis¹⁴. The total cumulate ultramafic and

transition zone rocks are estimated to be 60% olivine(Fo_{85}) + 30% clinopyroxene($\text{En}_{50}\text{Fs}_7\text{Wo}_{43}$) + 10% plagioclase(An_{80}). From these chemical constraints, the bulk composition of the oceanic crust is estimated in Table 1. (The MgO contents can be expected to vary locally, probably from ~16 to ~20% MgO, due to the variable histories and parameters of partial melting and melt extraction.) This very magnesian composition (~18% MgO) of the oceanic crust requires that the parental magma for ocean floor basalts be equally very magnesian.

Magma generation in the upper mantle

It is widely accepted that the massive residual harzburgites found at the base of many ophiolites are the result of partial melting and extraction of magma from the upper mantle^{15,16}. As such, these harzburgites must have been in equilibrium with the parental magma of ocean floor basalts and place important constraints on the chemistry of this parental magma.

Petrologically, the harzburgites have a relatively constant olivine to orthopyroxene ratio of 80:20 with trace amounts of spinel and clinopyroxene^{15,16}. The clinopyroxene in the harzburgite has exsolved from orthopyroxene during re-equilibration in the upper mantle or has crystallised from trapped interstitial melt which was not extracted from the upper mantle^{15,16}. As such, clinopyroxene was eliminated as a residual phase during the melting event in the upper mantle and the primary melt must have been in equilibrium with olivine(Fo_{91}) and orthopyroxene(En_{91}), as these are typical compositions of residual minerals in upper mantle harzburgites^{15,16}.

The residual harzburgites from several ophiolite and alpine peridotite terrains are remarkably similar in their modal proportions, mineral chemistry, and bulk compositions^{15,16}, indicating that the process of magma generation in ophiolites and the oceanic crust is relatively uniform, with melting continuing until the magma has ($\text{Mg}/\text{Mg} + \text{Fe}^{2+}$) ~0.77 and is multiply saturated with olivine and orthopyroxene as liquidus phases at the pressure of final equilibration, that is, 5–10 kbar.

Melting of lherzolite compositions must progress to high melt increments to eliminate clinopyroxene and spinel resulting in melt–solid equilibria involving only olivine, orthopyroxene, and a MgO-rich liquid. Mysen and Kushiro¹⁷ have shown that such melts contain 19.6% MgO, 9.7% FeO^* , and 49.6% SiO_2 at 20 kbar with 28.5% partial melting. Boettcher *et al.*¹⁸ have produced a similar liquid by melting a spinel lherzolite at 20 kbar in the presence of CO_2 and H_2O . Similarly, high-pressure melting experiments on peridotitic komatiites¹⁹ suggest that at 10 kbar pressure, liquids in equilibrium with olivine and orthopyroxene contain 18–19% MgO.

Table 1 Lithologies used to calculate the composition of the oceanic crust

	Average FAMOUS glass	Average oceanic tholeiite glass	Average Tortuga sheeted dyke	Average Tortuga 'gabbro'	Computed TZ and ultramafic rocks	Calculated composition for oceanic crust
SiO_2	50.23§	50.68†	49.00‡	49.74‡	45.19	47.76
TiO_2	1.02	1.49	1.44	0.47	—	0.59
Al_2O_3	15.52	15.60	16.01	19.08	3.26	12.06
Cr_2O_3	0.05	—	0.05	0.10	—	—
FeO^*	9.17	9.85	11.12	5.50	9.99	8.96
MnO	0.15	—	—	0.10	—	0.12
MgO	9.11	7.69	7.81	8.89	32.91	17.78
CaO	11.89	11.44	11.77	14.36	8.21	11.20
Na_2O	2.31	2.66	1.91	1.79	0.44	1.31
K_2O	0.14	0.17	0.08	0.02	—	0.03
Total	99.59	99.58	99.19	100.05	100.00	99.81

Note the similarity between FAMOUS, average oceanic tholeiite, and Tortuga compositions. The cumulate transition zone (TZ) and ultramafic assemblages are calculated assuming 60% (Fo_{85}) + 30% ($\text{En}_{50}\text{Fs}_7\text{Wo}_{43}$) + 10% (An_{80}). See text for details.

§ Ref. 13.

† Ref. 12.

‡ D. E., unpublished.

Can the fractionation of basalts (~10% MgO) generate the oceanic crust?

It is generally accepted that the pseudostratigraphy of oceanic crust is a consequence of igneous processes operating at mid-oceanic ridges. The major operating processes are magma intrusion and eruption at shallow crustal levels and gravitational crystal accumulation in large magma chambers at deeper crustal levels. Hence, a prerequisite of melt ascending from the mantle is that it be capable of reproducing the observed crustal lithologies and the cryptic variations within them. The basaltic glasses recovered in the FAMOUS area are among the most magnesian glass samples recovered from the ocean floor^{13,20}. Their high *mg* values (0.66–0.72) and high nickel contents (100–260 p.p.m.) led Langmuir *et al.*²⁰ to suggest that they represent primary partial melts of the upper mantle. If this interpretation is correct, then, with reference to the discussion above, fractional crystallisation of these basalts should be capable of generating the petrochemical characteristics of the oceanic crust as shown in Fig. 1.

If we use this ophiolite constrained petrological model (Fig. 1), one constraint placed on the primary magma is that it must be capable of fractionating 10% Fo₉₀ (representing the cumulate dunites) followed by fractionation of cumulate pyroxene bearing and transition zone lithologies and still be capable of extruding an ocean floor basalt from the upper portions of the crustal level magma chamber. FAMOUS glass 527-1-1 is an example of a basalt proposed as a primary mantle melt^{20,21}. Extraction of

10% olivine (Fo₉₀) from a melt of this composition (Liq₁) produces a residual liquid (Liq₂) (Fig. 2). The daughter melt (Liq₂) has a very low MgO content and *mg* value and is incapable of fractionating further to give the remaining ultramafic and mafic cumulate sequence. Thus it is concluded that basaltic melts with 9–11% MgO are incapable of producing the observed crustal lithologies and cannot be the ultimate parent to more evolved ocean floor basalts. Additionally, the recent data of Bender *et al.*²², demonstrate that 527-1-1 has undergone at least 10% fractionation of olivine and that the composition of 527-1-1 has been modified to the extent that <2% (by weight) of olivine fractionation will mark the onset of plagioclase crystallisation.

The above discussion suggests that a substantially more magnesian liquid may satisfy the petrological constraints. Hence, a similar calculation has been performed for NT-23, an MgO-rich dyke from the Tortuga complex with the results tabulated in Fig. 2. In this case, the daughter liquid remains highly magnesian (MgO = 14%, *mg* = 72) after 10% olivine (Fo₉₀) removal. Cumulate pyroxenite and transition zone minerals indicate a further removal of about 20% of olivine, clinopyroxene, and plagioclase in the ratio 40:45:15 occurs. The residual liquid (Liq₃) still has a slightly higher MgO content than FAMOUS glass 527-1-1, but is close in composition to the latter. The observed crustal lithologies and cryptic chemical variations can be best modelled by fractionation of highly magnesian melt with ~18% MgO. During this process, residual liquids approach the composition of ocean floor tholeiites,

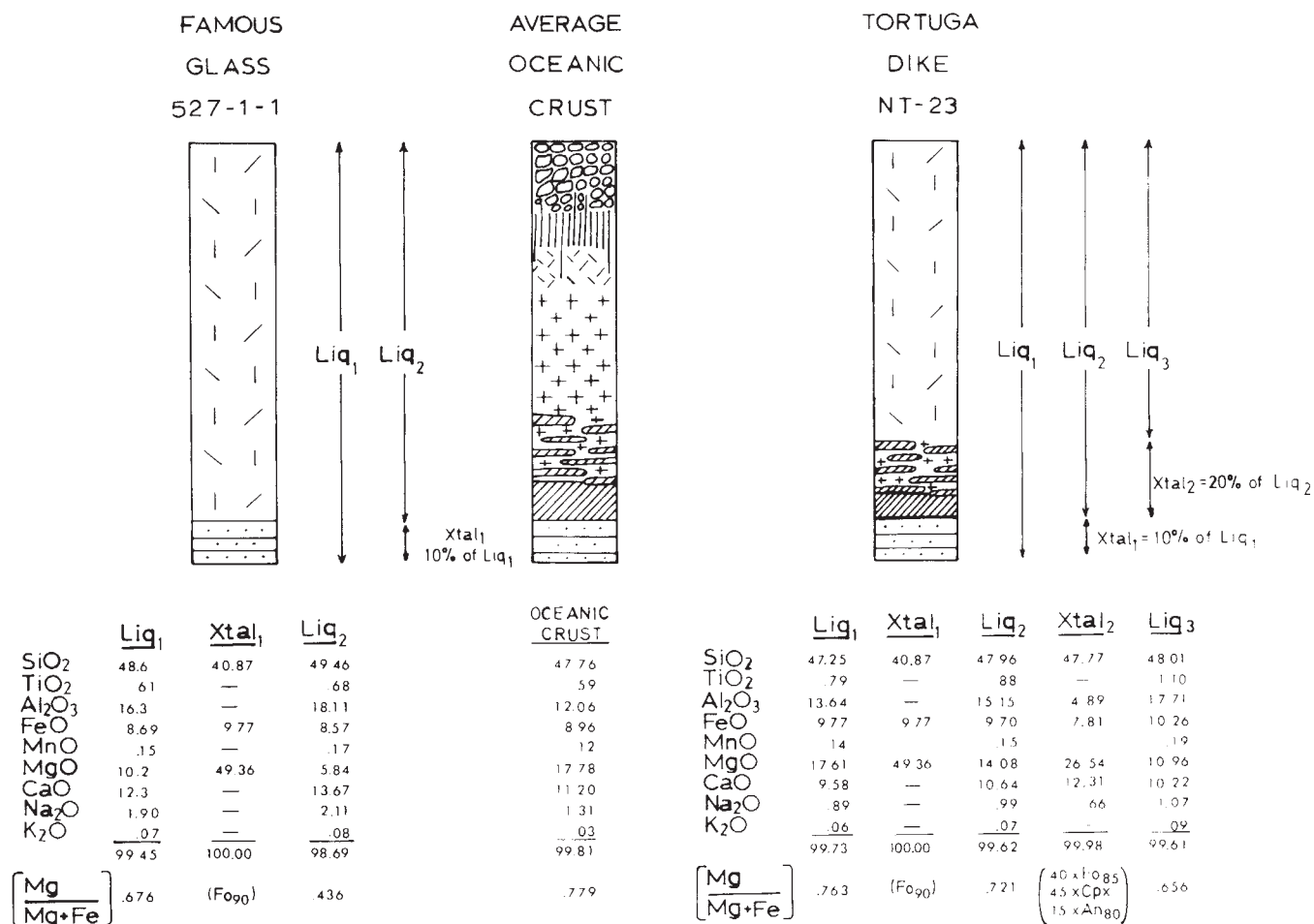


Fig. 2 Diagram demonstrating the results of fractional crystallisation of an ocean-floor tholeiite (FAMOUS glass) versus a high magnesian basalt (NT-23). The schematic columnar sections demonstrate that the FAMOUS glass is incapable of generating the petrological features of the oceanic crust, while fractionation of NT-23 is capable of reproducing these chemical features. See text for details.

including the most magnesian FAMOUS basalts, so that magmas erupted along mid-oceanic ridges are strongly evolved, secondary liquids which have been derived by almost 30% fractional crystallisation from the primary magma.

Flux of magma through the oceanic crust

Most models for the structure of oceanic crust at mid-oceanic ridges incorporate a large magma reservoir within the crust. Although direct evidence is lacking, thick cumulate sequences in ophiolites are ubiquitous and must represent the sites of former, volumetrically large magma chambers. These cumulate lithologies imply reservoirs ~5 km in vertical thickness situated just above the residual harzburgite, an estimate consistent with geophysical measurements¹⁰. The width of sea floor over which eruptions occur reflects a reasonable value for the width of the magma chamber. Measurements are scarce, but in the FAMOUS area basalts were erupted over the entire 6 km width of the median valley¹³. Therefore, a magma reservoir beneath an active ridge may have a volume of about 30 km³ (3×10^{10} m³) per km of ridge length.

If the magma chamber maintains a steady state, the volumetric addition of melt to the magma reservoir from the mantle is the product of spreading rate (~2 cm yr⁻¹) and crustal thickness (7 km), that is 1.4×10^5 m³ per yr per km of ridge length. This volume is balanced by the extrusion of $\sim 0.4 \times 10^4$ m³ yr⁻¹ of magma from the top of the reservoir per km of ridge length. However, the important consideration is that the flux of magma into and out of the magma reservoir is extremely small compared with the volume of the reservoir. (Nisbet and Fowler²³ have reinterpreted the data from the FAMOUS area to exclude any volumetrically large magma chamber from operating in the area at present. However, even if the estimate presented here is two orders of magnitude too high, the conclusions presented here remain the same.) Consequently, the addition of melt from the mantle has no measurable effect on the overall chemistry of the magma chamber. Any variation in the composition of mantle-derived melts entering the chamber will also be undetectable and it seems highly unlikely that such variations could be observed in surface eruptions. More likely, the compositional variations observed in erupted basalts²⁰ reflect fractional crystallisation and magma mixing within the crustal magma reservoir and are the result of crustal processes rather than dynamic mantle melting.

However, highly magnesian, aphyric lavas have been described from Baffin Island^{24,25} and Svartenhuk^{24,25}, and have recently been discovered as dykes in the Tortuga ophiolite

complex, Chile²⁶, indicating that occasionally such primary melts do reach the near-surface environment. As it seems unlikely that such magmas can ascend uninterrupted once a steady-state magma chamber has developed they must erupt before the reservoir development or in the dying stages of activity when the reservoir has largely solidified. The former situation may arise during the earliest stages of continental rifting and would fit the tectonic position of the Baffin Bay lavas²⁴. The latter situation may be the instance for the Tortuga dykes.

High magnesia dykes from Tortuga

Table 2 shows the compositions of four MgO-rich dykes which intrude the cumulate rocks in the Tortuga ophiolite complex. Textural and petrological evidence indicates that these dykes were intruded as liquids²⁶. The similarity of NT-23 to the Group II olivine basalts of Baffin Island and Svartenhuk²⁴ as well as the average oceanic crust (Table 1) indicates that this is a primary mantle melt and the parental magma for ocean floor basalts.

In conjunction with the data and observations of Donaldson and Brown²⁷ and Rhodes and Dungan²⁸, it is critical to evaluate the role of high calcium picritic magmas in the fractionation scheme producing ocean floor basalts. The similarities of NM-5 with Donaldson and Brown's²⁷ melt inclusions and Rhodes and Dungan's²⁸ calculated parental magma is surprising in view of the diverse methods from which these values have been derived. The gradual build-up of calcium in the magma during fractional crystallisation of the primary melt culminates in high calcium picritic liquids with ~12–14% MgO. Thus, a liquid line of descent from primary mantle melts (18% MgO) through high-calcium picrites (for example, NM-5) towards oceanic tholeiites is indicated by the compositions of these Tortuga dykes.

Conclusions

The present discussion has demonstrated the significance of highly magnesian basalts in the formation of the oceanic crust. Correlatively, ocean-floor tholeiites (9–11% MgO) cannot be accepted as primary mantle melts. High-temperature crystallisation experiments clearly indicate that oceanic tholeiites are multiply saturated with combinations of plagioclase, olivine, and clinopyroxene at low pressures^{2,3} as a consequence of low pressure fractionation. O'Hara^{2,3} has repeatedly used this powerful argument against the primary nature of oceanic tholeiites.

As a result of the steady-state magma chamber operating in the oceanic crust, O'Hara²⁹ conceptualised several characteristics of magmas in the oceanic crust which are controlled and modified by the magma chamber itself. Consequently, there remains a distinctive compositional gap between the magma segregating from the upper mantle and oceanic tholeiites with 9–11% MgO. Because this gap represents the effects of extensive fractional crystallisation and magma mixing, caution must be exercised when evaluating mantle chemistry and processes using evolved compositions.

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1. Bowen, N. L. *The Evolution of the Igneous Rocks* (Princeton University Press, 1928).
2. O'Hara, M. J. *Scott. J. Geol.* **1**, 19–40 (1965); *Earth Sci. Rev.* **4**, 69–133 (1968).
3. O'Hara, M. J., Saunders, M. J., & Mercy, E. L. *P. Phys. Chem. Earth Inter.* **9**, 571–604 (1975).
4. Kidd, W. S. F., Dewey, J. F. & Bird, J. M. *Can. J. Earth Sci.* **15**, 781–804 (1978).
5. Dewey, J. F. & Kidd, W. S. F. *Geol. Soc. Am. Bull.* **88**, 960–968 (1977).
6. Coleman, R. G. *Ophiolites* (Springer, Berlin, 1977).
7. Jackson, E. D., Green, H. W. & Moores, E. M. *Geol. Soc. Am. Bull.* **86**, 390–398 (1975).
8. Laurent, R. *North American Ophiolites*, (eds Coleman, R. G. & Irwin, W. (in the press)).
9. Church, W. R. & Riccio, L. *Can. J. Earth Sci.* **14**, 1156–1165 (1977).
10. Christensen, N. I. & Salisbury, M. H. *Rev. Geophys. Space Phys.* **13**, 57–86 (1975).
11. Stern, C. R. & Elthon, D. *Tectonophysics* (in the press).
12. Melson, W. G., Vallier, T. L., Wright, T. L., Byerly, G. & Nelson, J. *Geophysics of the Pacific Ocean Basin* (Am. Geophys. Un., Washington, 1976).

Table 2 Compositions of high magnesia Tortuga dykes

	NT-23	NT-20	PII*	NM-5	Melt inclusions†	Rhodes-Dungan parental magma‡
SiO ₂	47.25	47.70	49.13	50.83	50.26	—
TiO ₂	0.79	0.58	0.66	1.06	0.61	0.6–0.9
Al ₂ O ₃	13.64	14.36	16.38	14.64	14.21	—
Cr ₂ O ₃	0.20	0.12	—	0.05	0.10	—
FeO*	9.77	11.59	8.79	7.89	6.82	6.5–7.0
MnO	0.14	0.17	0.15	0.14	0.10	—
MgO	17.61	14.78	11.85	10.97	11.89	10
CaO	9.58	10.76	11.54	13.11	13.46	12.0–13.5
Na ₂ O	0.89	1.41	1.60	1.41	1.42	2.0
K ₂ O	0.06	0.14	0.04	0.14	0.07	—
P ₂ O ₅	0.07	0.02	0.06	0.07	—	—
Total	100.00	100.63	100.47	100.31	98.66	—

Note the gradual increase in calcium in the magma during fractionation of the primary melt (NT-23) resulting in high calcium picrites similar to the estimates of the parental magma for ocean floor tholeiites by Donaldson and Brown²⁷ and Rhodes and Dungan²⁸. All of the Tortuga samples are fine to very fine grain and have been altered to variable extents. Cpx (~En₄₄Di₄₂Fs₁₄); Plag (~An₇₀) Ilmenite, and Picotite (Al₂O₃ = 30–40%, FeO = 24–26%, Fe₂O₃ = 1–2%, Cr₂O₃ = 23–31%, MgO = 4–9%) are observed in subophitic to intersertal textures. NT-23 contains a few per cent of plagioclase megacrysts.

* Most magnesian dyke reported by Stern³⁰.

† Average of 13 melt inclusions in spinels²⁷.

‡ Estimated composition of the parental magma for ocean floor basalts²⁸.

13. Bryan, W. B. & Moore, J. G. *Geol. Soc. Am. Bull.* **88**, 556–570 (1970).
14. England, R. N. & Davies, H. L. *Earth planet. Sci. Lett.* **17**, 416–425 (1973).
15. Menzies, M. *Oregon Dept. Geol. Miner. Indust. Bull.* **96**, 129–148 (1977).
16. Dick, H. J. B. *Am. J. Sci.* **277**, 801–832 (1977).
17. Mysen, B. O. & Kushiro, I. *Am. Miner.* **62**, 843–856 (1977).
18. Boettcher, A. L., Mysen, B. O. & Modreski, P. J. *Phys. Chem. Earth Inter.* **9**, 855–867 (1975).
19. Bickle, M. J., Ford, C. E. & Nisbet, E. G. *Earth planet. Sci. Lett.* **37**, 97–106 (1977).
20. Langmuir, C. H., Bender, J. F., Bence, A. E., Hanson, G. N. & Taylor, S. R. *Earth planet. Sci. Lett.* **36**, 133–156 (1977).
21. Hodges, F. N. & Bender, J. F. *Geol. Soc. Am. Prog. Abstr.* 920 (1976).
22. Bender, J. F., Hodges, F. N. & Bence, A. E. *Earth planet. Sci. Lett.* **41**, 277–302 (1978).
23. Nisbet, E. G. & Fowler, C. M. R. *Geophys. J. R. astr. Soc.* **54**, 631–660 (1978).
24. Clarke, D. B. *Contr. Miner. Petrol.* **25**, 203–224 (1970).
25. O'Nions, R. K. & Clarke, D. B. *Earth planet. Sci. Lett.* **15**, 436–446 (1972).
26. Elthon, D. *Geol. Soc. Am. Prog. Abstr.* 396 (1978).
27. Donaldson, C. H. & Brown, R. W. *Earth planet. Sci. Lett.* **37**, 81–89 (1977).
28. Rhodes, J. M. & Dungan, M. A. *Sec. Ewing Symp. Abstr.* 27–28 (1978).
29. O'Hara, M. J. *Nature* **266**, 503–507 (1977).
30. Stern, C. R. *Contr. Miner. Petrol.* (in the press).

Palaeoclimatic inferences from former glaciers in Scotland and the Lake District

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Firn line altitudes of Loch Lomond Advance glaciers in Scotland and the Lake District imply that snowfall was associated mainly with south to southeasterly air streams preceding fronts. Precipitation was high in the south-west Grampians and very low in the north-west Cairngorms and Speyside. The junction of polar and relatively warm ocean waters at the latitude of south-west Ireland was associated with many depressions following more southerly tracks than at present. It is suggested that during ice-sheet growth the zone of maximum snowfall moved southwards over the British Isles.

THE last ice-sheet that existed in the British Isles is generally believed to have attained its greatest extent ~17,000 BP, when its southern margin was located in the north-west Midlands. Radiocarbon dating of the basal organic material in present or former lake sites shows, if the dates are valid, that extensive areas of the Scottish Highlands had become ice-free by ~13,000 BP¹. By this time the polar water that had surrounded the British Isles had been replaced by much warmer water². This suggests that the remains of the ice-sheet would have melted rapidly, the British Isles becoming ice-free probably between 13,000 and 12,500 yr BP.

However, the polar waters subsequently spread southwards again, extending as far as the latitude of south-west Ireland³. This was accompanied by a return to cold conditions. This cold period—the Loch Lomond Stadial (equivalent to the Younger Dryas of Scandinavia)—occurred around 11,000–10,000 yr BP (more exact dating is being discussed). This paper makes climatic inferences from the glaciers that developed at this time.

Scotland

Figure 1 shows the limit of the Loch Lomond Advance in Scotland, although evidence is lacking in some areas, most notably in parts of the western Highlands, where a broken line is shown. Figure 2 shows generalised equilibrium firn lines for the former glaciers in the Highlands and Inner Hebrides based on 226 data points. Firn lines were calculated from altitude/area distributions of the glacier surfaces, using a method described elsewhere⁴.

By far the largest mass of ice was in the western Highlands southwards from the latitude of Skye (Fig. 1). This seems to suggest that snowfall was associated mainly with air streams from the west or south-west. However, superimposed on this pattern is another one: this is the contrast between north- and south-facing glaciers in many individual upland areas. For

example, on the external slopes of the Cuillin Hills of Skye (A in Figs 1 and 2) there is a continuous line of cirques facing directions that change from south-southwest through west to north-east. One might expect, from the influence of direct insolation, north-facing glaciers to have been larger and to have extended to lower altitudes than south-facing ones. However, the converse applied: the combined volume of the two glaciers that faced north and north-east was only one-third of that of the two glaciers that faced south-southwest, and the former had firn lines 300 m higher than the latter⁵.

In the central Grampians on the high ground on both sides of the Pass of Drumochter (through which run the Perth–Inverness railway and main road (A9)) ice built up to form an ice-cap, whose outlet glaciers flowed into the pass and down many other valleys (B in Figs 1 and 2). The ice-cap was asymmetrical in form, the bulk of it lying south of the east–west axis of the high ground. Equilibrium firn lines rose northwards, from below 700 m on the southernmost parts of the ice-cap to 800 m or more on its northern parts.

In the western Cairngorms (C in Figs 1 and 2) the largest glacier flowed southwards. In contrast, in the northern part of the area only three north-facing cirques contained glaciers, all of which were very small. Firn lines rose from below 800 m in the south to over 1,000 m in the north⁶.

The regional firn line in north-west Scotland increased in altitude from the coast to the interior (Fig. 2). This overall trend masks the detailed pattern in some individual upland areas. For example, in the Ben More Assynt Range, in the Fannich mountains, and in the mountain mass culminating in Ben Dearg, southward-flowing glaciers were larger, sometimes very much larger, than those that faced northwards⁷.

All the specific areas mentioned above have a north–south extent of no more than 25 km. Hence regional temperature differences cannot be invoked to explain the north–south contrasts. In fact, temperature differences related to altitude would have favoured the higher, north-facing glaciers in that here a higher proportion of the annual precipitation would have fallen as snow. Furthermore, the influence of direct insolation opposes the pattern described above. Hence some major factor must have been over-riding these influences. I suggest the only possible explanation is much higher precipitation on the southern sides of individual upland areas than on their northern sides. This in turn implies that the principal snow-bearing air streams were from southerly directions.

The firn lines in the south-east part of the Grampian Highlands, corrected for the influence of direct insolation (unpublished data), trend almost exactly parallel with the Highland Fault, rising northwestwards from 500 m to over 800 m (D in Fig. 2). Along the fault there is an abrupt increase of

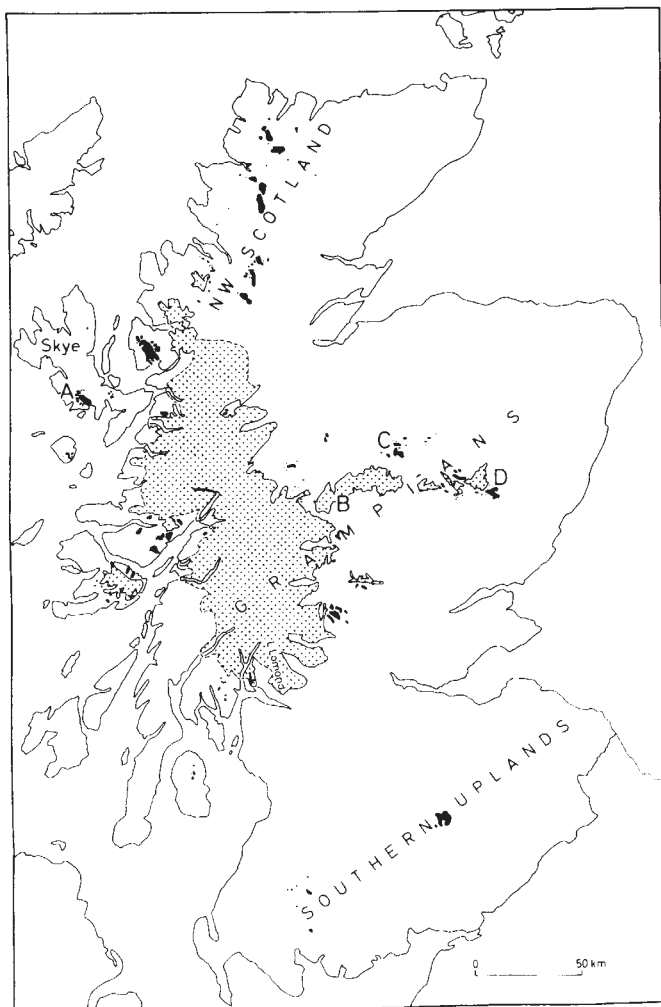


Fig. 1 Limit of the Loch Lomond Advance in Scotland. The map includes unpublished information provided by C. K. Ballantyne, R. Cornish, M. Robinson, D. G. Sutherland, P. Thorpe and T. Wain-Hobson. For reference letters see text.

several hundred metres in ground altitude. The firn lines imply that, at any given altitude, snowfall was heaviest along the Highland Edge and diminished thence northwestwards into the interior of the Highlands. This indicates uplift of air streams as they crossed the Highland Edge. Such uplift could not have occurred with southwesterly air streams, for they would have flowed parallel with the Highland Edge: with snow-bearing winds from southerly directions, it must have been south to southeasterly air streams that brought the principal snowfalls to the south-east Grampians.

Lake District

The Lake District differs from the preceding examples in that there is no south to north rise in firn line altitudes (unpublished data). In fact there is no significant trend in any direction. A major factor here was snow transfer to glaciers from surrounding higher ground by wind. Potential snow-blowing areas were measured on Ordnance Survey maps for each glacier and each quadrant, using rules specified elsewhere⁸. Each snow-blowing area was divided by its associated glacier area to give a snow-blowing ratio. Regression of firn line altitudes on snow-blowing ratios for the north-west quadrant for all glaciers indicated no significant relationship. This applied also to the north-east quadrant. For the south-west and south-east quadrants, however, there are highly significant inverse relationships. Thus some glaciers were relatively low because of snow blown from

the two latter directions and this distorts the distributional pattern of firn line altitudes.

Snow-blowing would have been most important during and immediately after snowfall, so that the above relationships again point to the importance of snowfall with southerly winds. The synoptic situation implied is one in which warm or occluded fronts approached from the west or south-west, the major snowfalls occurring with south to southeasterly winds before fronts arrived, succeeded by further snowfalls with moist south to southwesterly air streams after fronts had passed. Frequent repetition of this situation necessitates many depressions following more southerly tracks than are normal at the present day, this in turn being related to the junction of polar and relatively warm ocean waters at the latitude of south-west Ireland.

The largest glaciers in the Lake District were along the east-west mountain axis, especially in the central mountain area. This reflects the strong influence of relief on precipitation, as at present. However, despite much high ground, glaciers were very small in the north-west (north and north-east of Ennerdale): and size alone exaggerates their importance, for all of them occupied locations relatively sheltered from direct insolation and many have high snow-blowing ratios for the south-east and/or south-west quadrants. This implies relatively low precipitation in the north-west Lake District and points to southerly air streams, especially south to southeasterly ones, as having been associated with the principal snowfalls, the north-west being in the precipitation shadow of the central mountain area.

Snowfalls and glacier growth

Although south to southeasterly air streams were associated with the major snowfalls in the Highlands, moist southwesterly air streams would have been increasingly important providers of

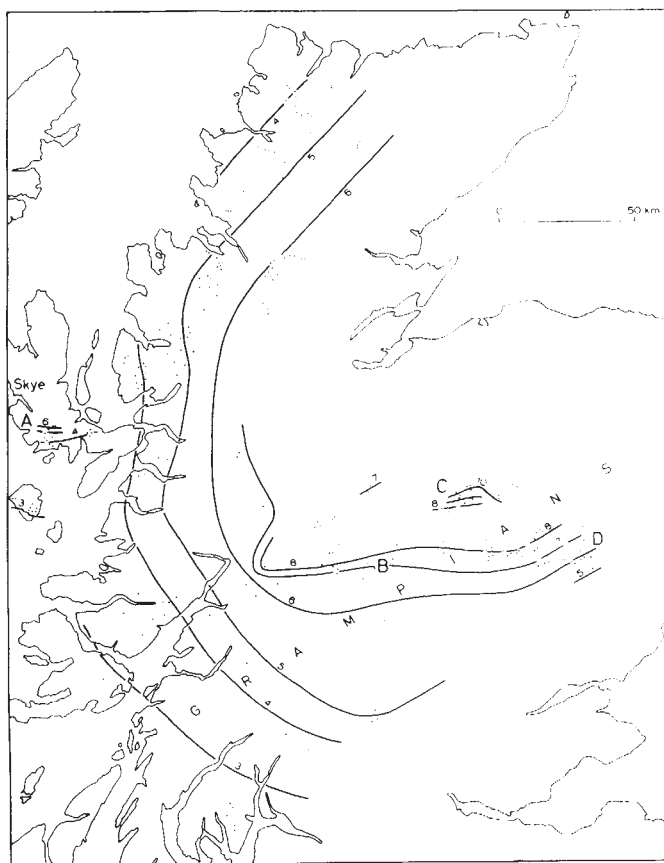


Fig. 2 Equilibrium firn line altitudes for Loch Lomond Advance glaciers in the Highlands and Inner Hebrides. Altitudes in hundreds of metres. For reference letters see text.

precipitation towards the west. Hence the largest ice mass developed in the west (favoured also by relief) and the regional firn line was lowest (300 m) in the south-west Grampians, which was most exposed to air streams from both directions. Present precipitation in the former glacier accumulation areas of the south-west Grampians is 3,000–4,000 mm. In stadial times it would have tended to be less due to lower air temperatures, but more due to the much more vigorous interaction of air masses⁸. Assuming these two tendencies roughly cancelled each other, we may obtain an indication of former summer temperatures using Liestøl's graph (Fig. 3). Allowing for 20–25% summer rain, this graph indicates that with a summer temperature of $3.5 \pm 0.5^\circ\text{C}$ the range of annual precipitation covered is 2,700–4,000 mm. I suggest this considerable range includes all reasonable possibilities. Using the 400 m firn line this is equivalent to a temperature at sea level (May–September) of 6.0°C , or a July temperature of 7.5°C . As these temperatures are derived from firn lines for glaciers in equilibrium, temperatures during the stadial would have been slightly lower.

In the Grampians the firn line rose 700 m (Fig. 2). That this rise was due mainly to precipitation differences rather than to temperature differences is indicated by the great contrasts in glacier dimensions. For example, in the south-west Grampians the Loch Lomond glacier alone had a volume of about 80 km^3 , whereas the combined volume of the 17 glaciers that existed in the Cairngorms, despite much higher altitudes, was only 1.7 km^3 . The glaciers in the north-facing cirques of the north-west Cairngorms were tiny and four cirques at 1,000 m altitude failed to nourish glaciers, suggesting extremely low precipitation here. An indication of the amount may be obtained as follows.

Assuming 750 yr for glacier growth, the average annual net accumulation on the Loch Lomond glacier above the equilibrium line was slightly over 600 mm (water equivalent). For the three north-facing Cairngorm glaciers the values are 100–150 mm. This suggests that precipitation on the Loch Lomond glacier was 4–6 times that of the three Cairngorm ones. Even taking the maximal value of 4,000 mm annual precipitation for the south-west Grampians proposed above, this implies only about 800 mm in the north-west Cairngorms. However, even this low value seems to be too high, for the following reasons. (1) During much of the time it was advancing the Loch Lomond glacier would have been subject to loss through calving in the ablation season. (2) This glacier flowed southwards whereas the three Cairngorm glaciers flowed northwards. (3) The latter were backed by plateaus from which snow would have been blown onto them by southerly winds. (4) The above estimate includes an allowance of 20–25% for summer rain, which seems reasonable for the south-west Grampians but is almost certainly too high for the high-altitude glaciers of the north-west Cairngorms. Taking these four factors into account, I suggest that annual precipitation at an altitude of 1,000 m in the north-west Cairngorms may well have been only 500–600 mm. At the much lower altitude of the floor of the Spey Valley 200–300 mm seems a reasonable estimate. Birks and Mathewes⁹ concluded from pollen analyses that the Spey Valley had "a rather arid climate" during the Loch Lomond Stadial.

Figure 1 also shows the very small area covered by glaciers in north-west Scotland. This partly reflects the much smaller area of high ground compared with further south in the Highlands. This is not apparently the sole cause, however. The average firn line altitude for 68 glaciers in north-west Scotland is 520 m. For 64 glaciers in the Lake District it is 540 m. As these two areas differ in latitude by 400 km, one might reasonably expect a much lower average firn line in north-west Scotland due to the influence of temperature. The small difference therefore suggests that precipitation was considerably lower in north-west Scotland than in the Lake District. The former probably received much of its precipitation from occasional depressions whose centres normally passed to the north-west of the area as they tracked northeastwards. But the majority of depressions took more southerly tracks (related to the junction of cold and relatively warm ocean waters) and this seems to have been the

basic cause of the precipitation deficiency in north-west Scotland.

Ice-sheet accumulation

This interpretation seems relevant to ice-sheet accumulation. It is known that when the last ice-sheet existed, the Southern Uplands of Scotland were a major centre of ice accumulation. In the west the Southern Uplands ice at first flowed northwards, despite the opposition of Highland ice, before the latter deflected it. The Southern Uplands ice extended nearly to the site of Edinburgh and it produced the major assemblage of ice-moulded features in the Tweed basin. However, during the Loch Lomond Stadial there were only paltry glaciers in the Southern Uplands. Some of these glaciers are not shown in Fig. 1 because details are not available, but the mapping is complete in the west (Loch Doon Basin and vicinity), which had previously been a major centre of ice-sheet radiation.

The question thus arises: why were ice-sheet conditions so different from those in the Loch Lomond Stadial? A lower firn line during ice-sheet build-up than during the stadial would have allowed glaciers to accumulate more widely in the Southern Uplands. However, a lower firn line would also have benefited the Highland ice. If we regard Fig. 1 as portraying a stage in ice-sheet growth, the lowering of the firn line would probably have caused the Highland ice to overwhelm the Southern Uplands before they could develop a large ice mass.

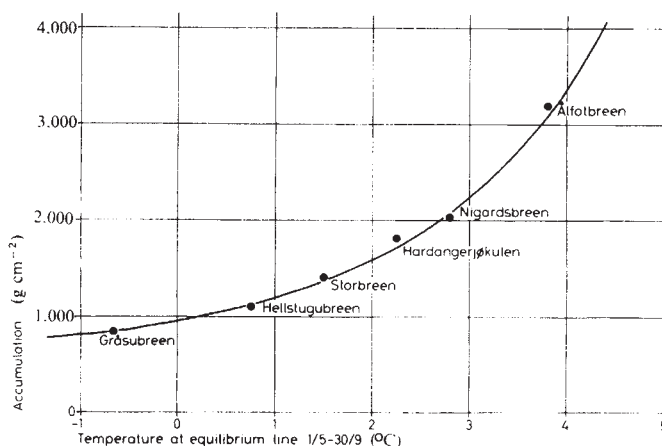


Fig. 3 The relationship between accumulation (winter balance) and summer temperature at the firn line for certain Norwegian glaciers. Compiled by O. Liestøl.

Conclusions

As the oceanic polar front moved southwards during ice-sheet growth, and with it the polar front in the atmosphere, I suggest that the zone of maximum precipitation also moved southwards. During the Loch Lomond Stadial, precipitation seems to have been deficient in the northern mainland of Scotland. We may envisage the next stage as a decrease in precipitation over the main Highland ice mass and an increase over the Southern Uplands. This idea can also explain why the Lake District, with only 3.3 km^3 of ice during the Loch Lomond Stadial, was an important centre of ice radiation in ice-sheet conditions. Continued southwards movement of the zone of maximum precipitation can explain why the Welsh uplands, where stadial glaciers were tiny, had an extensive plateau ice-cap feeding into the ice-sheet.

If this idea is valid one consequence is as follows: Many maps of the glaciation of the British Isles have shown ice from the Scottish Highlands flowing down the Firth of Clyde and through the Irish Sea to reach south-west Wales and the English Midlands. Among the erratics that contribute to this picture are

the famous ones of Ailsa Craig. However, if the zone of maximum precipitation moved southwards as an ice-sheet built up, thus affecting the relative importance of different parts of the ice-sheet, one may envisage erratics having been transferred from one area of local ice dominance to the next, without the occurrence of the continuous flow apparently suggested by erratics.

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1. Sissons, J. B. & Walker, M. J. C. *Nature* **249**, 822–824 (1974).
2. Ruddiman, W. F. & McIntyre, A. *Quat. Res.* **3**, 117–130 (1973).
3. Ruddiman, W. F., Sancetta, C. D. & McIntyre, A. *Phil. Trans. R. Soc. B* **280**, 119–142 (1977).
4. Sissons, J. B. *Trans. Inst. Br. Geogr.* **62**, 95–114 (1974).
5. Sissons, J. B. *Scott. J. Geol.* **13**, 23–26 (1977).
6. Sissons, J. B. *Scott. geogr. Mag.* (in the press).
7. Sissons, J. B. in *Studies in the Scottish Lateglacial environment* (eds Gray, J. M. & Lowe, J. J.) 45–59 (Permagon, Oxford, 1977).
8. Sissons, J. B. & Sutherland, D. G. *J. Glaciol.* **17**, 325–346 (1976).
9. Birks, H. H. & Mathewes, R. W. *New Phytol.* **80**, 455–484 (1978).

Transmission of allosteric effects in DNA

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Binding of distamycin induces a cooperative transition of calf thymus DNA to a new form with higher affinity for the drug and altered structural properties.

A PROTEIN or other ligand bound to one section of DNA can affect the expression of genetic information in a different DNA segment by two general mechanisms. The first of these is direct physical interaction between one bound protein and another. For example, promoter and repressor binding sites may overlap, as in the *Escherichia coli lac* operon¹, so that binding of repressor interferes sterically with polymerase binding, producing negative control. Positive control by direct interaction could result from favourable contacts between polymerase and a bound activator.

The alternative possibility for exertion of control-at-a-distance is indirect influence of one bound protein on binding of another because of structural alteration of the DNA that intervenes between the two sites. We refer to this as transmission of allosteric effects through DNA, a mechanism which though purely conjectural is supported by enough indirect evidence to require its serious consideration. For example, two arabinose promoters whose polymerase binding sites are separated by 100 base pairs clearly interact². At the level of model studies the size of the DNA region excluded by binding of drugs such as actinomycin³ or ethidium⁴ is larger than the size of the drug molecule as measured along the helix axis. Therefore, the neighbour exclusion effect⁵ must be propagated through the DNA helix; a specific stereochemical mechanism has been proposed by Sobell *et al.*⁶. The clearest example of transmission of long-range allosteric effects in a polynucleotide is the demonstration by Pohl *et al.*⁷ that ethidium binding affects the balance between the high and low salt double-helical forms of poly d(GC). Drug binding at high salt concentration was markedly cooperative, implying interaction over hundreds of base pairs. Furthermore, Wells *et al.*^{8,9} have provided evidence for transmission of structural effects along DNA.

Our purpose here is to show that a naturally occurring drug, distamycin, alters cooperatively the properties of a natural double-helical DNA. The results are interpreted as an allosteric conversion of DNA to a form that exhibits increased distamycin binding, and imply that a length of hundreds of base pairs of the average DNA is altered by drug binding.

Cooperative binding of distamycin to calf thymus DNA

Distamycin is a basic oligopeptide which exhibits strong binding to DNA¹⁰. The interaction is only weakly ionic¹¹ and does not involve intercalation¹²; attachment probably occurs in the small groove^{12–14}. Circular dichroic spectral changes imply a substan-

tial DNA conformational change^{11,15}; viscosity measurements reveal a length increase and perhaps an increase in persistence length¹⁶. Binding occurs preferentially to AT-rich regions^{16,17}.

We used a phase partition technique to measure the equilibrium binding of distamycin to calf thymus and *E. coli* DNA. This method was chosen because aqueous solutions of distamycin are too unstable to allow equilibrium dialysis, and optical titrations require the questionable assumption of a linear relationship between optical change and extent of binding. Figure 1 shows the marked contrast in binding properties observed for the two DNA samples. Cooperative binding of distamycin to calf thymus DNA is indicated by the pronounced maximum which occurs at about one drug molecule bound per 30 base pairs. Much weaker binding (except in the limit as the extent of binding approaches zero) characterises *E. coli* DNA, the monotonically decreasing curve indicating that cooperative interactions are absent. A cooperative binding isotherm was also found for distamycin with calf thymus DNA in 1 M salt concentration (data not shown).

DNA length changes on binding

We used the method of transient electric dichroism of rod-like DNA molecules¹⁸ to measure apparent length changes that occur in DNA on distamycin binding. Figure 2a and d show such data for calf thymus and *E. coli* DNAs, again revealing a distinct difference between the two samples. The apparent length of calf thymus DNA molecules increases by about 13% at a ratio of one drug molecule bound per 30 base pairs, and decreases again as more distamycin is added. At the maximum of the curve the calculated length increase is about 14 Å per drug molecule, much greater than we observed for intercalating drugs (2–4 Å) by the same technique¹⁹. *E. coli* DNA, on the other hand, showed no detectable length increase in the same conditions (Fig. 2d). Other DNA samples, such as those from *Clostridium perfringens*, *Micrococcus luteus* or human placenta changed in length by only a few per cent (data not shown) without evidence for the local maximum observed for calf thymus DNA. The antibiotic netropsin produced length changes in *E. coli* and calf thymus DNAs closely similar to those found for distamycin (manuscript in preparation).

Ethidium as a probe of altered DNA structure

To obtain further evidence for an alteration of DNA structure by distamycin, we studied ethidium binding in the presence of distamycin. We found a striking change in the orientation of the bound ethidium chromophore (at $r_{\text{ethidium}} = 0.1$) in the presence of distamycin at $r_{\text{distamycin}} = 0.03$ (this r value corresponds to the maxima of both the distamycin binding isotherm and the distamycin-induced length increase).

The reduced dichroism, ρ , of a molecule orientated in an electric field is given by $\rho = \frac{2}{3}(3 \cos^2 \alpha - 1)\Phi$, where α is the angle between the transition moment and the orientation direction, and Φ is the fractional orientation of the sample^{20,21}. In the limit of infinite electric field, Φ approaches 1, and α can be calculated from the extrapolated value of ρ , ρ_∞ . The maximum negative dichroism occurs when $\alpha = 90^\circ$, yielding $\rho_\infty = -\frac{2}{3}$. Figure 3 shows the field dependence of the reduced dichroism of bound ethidium in the presence of distamycin at $r_{\text{distamycin}} = 0.03$. The much larger dichroism values observed in the presence of distamycin extrapolate to -1.5 , indicating that the ethidium chromophore is perpendicular to the helix axis. In the absence of distamycin, as we have reported¹⁹, the ethidium dichroism is considerably smaller, implying tilting of the intercalated chromophore out of the plane perpendicular to the helix axis.

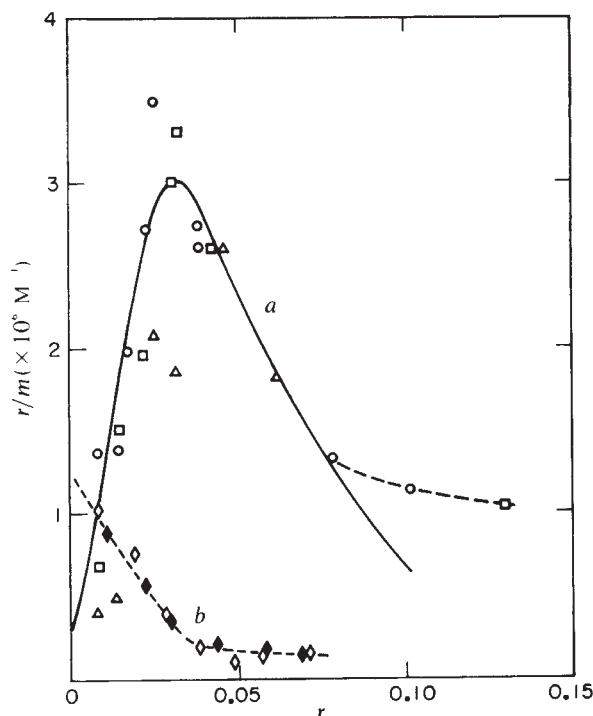


Fig. 1 Scatchard plot²⁴ of the binding equilibria of calf thymus (a) and *E. coli* (b) DNAs with distamycin at 11 °C in PES buffer (50 mM NaCl, 6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM EDTA, pH 6.5). Duplicate calf thymus (●, ○, □, △) and *E. coli* (◆, ◇) DNA samples were prepared by dissolving commercial (Sigma) DNA sample in 6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM EDTA, 1 mM Na cacodylate buffer and sonicating for 30 min at ice bath temperature with a Branson sonicator operated at full power. Following deproteinisation by repeated phenol extraction, samples were dialysed into 8 mM Na₂HPO₄, 2 mM NaH₂PO₄, 180 mM NaCl, 1 mM EDTA and fractionated on a Sepharose 4B column. The centre portion of the elution profile was pooled and dialysed into measurement buffer. Transient electric dichroism¹⁸ and gel electrophoresis against markers of *Hae*III restriction fragments of ColEI DNA²⁵ yielded 150 base pairs as the size of the calf thymus and *E. coli* samples used for measurement. Binding isotherms were measured by partitioning the drug between *n*-pentanol and an aqueous solution of DNA. 2 ml of *n*-pentanol was added to 2 ml of PES buffer containing DNA and distamycin A (Boehringer, Sigma, purified by LH 20 (Pharmacia) chromatography in CH₃OH/CHCl₃, 9/1 volume ratio) at known total concentrations. The mixture was shaken at 11 °C for 2 h, and the phases were allowed to separate. The free distamycin concentration (m) was determined from the absorbance of the organic phase ($\epsilon_{306} = 42,400 \text{ M}^{-1} \text{ cm}^{-1}$), using the partition coefficient $K = 19.5$ (organic/aqueous) determined in the absence of DNA. The total distamycin concentration (free + bound) was measured by dissociating the aqueous complex through addition of an equal volume of dimethyl sulphoxide (DMSO) (ϵ_{306} in PES/DMSO, 1:1 volume ratio = $32,800 \text{ M}^{-1} \text{ cm}^{-1}$). The bound drug concentration was calculated from the difference of free and total concentrations, and divided by the DNA base pair concentration to obtain r , the number of drug molecules bound per base pair. The solid curve (a) was calculated from a statistical mechanical theory (see text) of cooperative allosteric transition of calf thymus DNA from its initial form to another structure that has higher affinity for distamycin (manuscript in preparation).

Figure 2b, c, e, f show the results obtained from experiments similar to that in Fig. 3 but at different $r_{\text{distamycin}}$ values ($r_{\text{ethidium}} = 0.1$). The ethidium orientation angle (b) reaches a maximum at nearly the same $r_{\text{distamycin}}$ value for which the length increase is maximal. The calf thymus DNA length increase due to distamycin in the presence of constant ethidium (c) is only slightly smaller than observed in absence of ethidium (a). The control sample of *E. coli* DNA showed no variation in length or ethidium orientation angle as a function of distamycin concentration (e, f). The dichroism, ρ_∞ , of ethidium bound to *E. coli* DNA was very similar to the value found for ethidium bound to calf thymus DNA in the absence of distamycin.

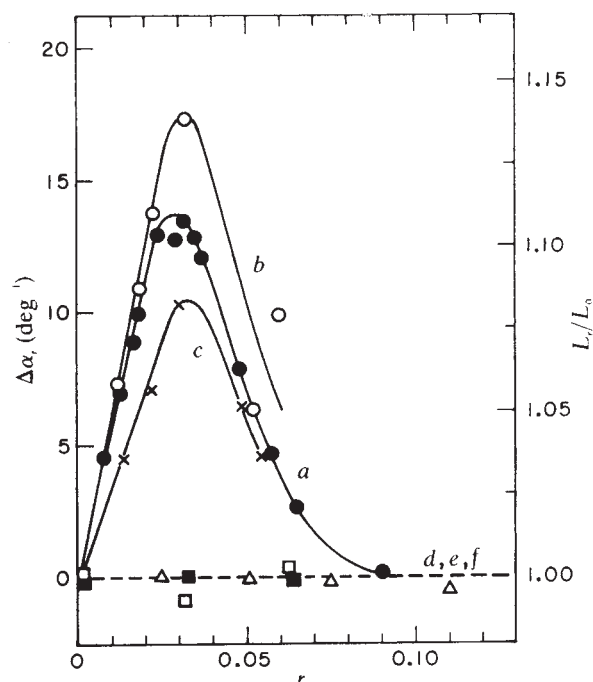


Fig. 2 Apparent fractional length (L) changes (a, c, d, f) and ethidium orientation angle (α) changes (b, e) as a function of degree of distamycin binding for calf thymus (a, b, c) and *E. coli* (d, e, f) DNA samples, prepared as described in the legend to Fig. 1 and dissolved in dichroism buffer (0.5 mM Na₂HPO₄, 1 mM NaH₂PO₄, 0.25 mM Na₂EDTA, pH 7.0) at 11 °C. Additional samples of calf thymus DNA were obtained by proteinase K (Merck) digestion and phenol extraction of mononucleosomes isolated from HI-depleted chromatin²⁶. These samples gave results identical to those obtained with sonicated samples. Samples a and d contain no ethidium, whereas samples b, c, e and f contain a constant amount of bound ethidium, $r_{\text{ethidium}} = 0.1$. Lengths were calculated from the dichroism rise time τ and Broersma's equation²⁷ for the rotational diffusion constant of a cylinder, which we earlier verified for rod-like DNA molecules¹⁸. We assumed no change in helix diameter on drug binding, an assumption justified by the logarithmic dependence of τ on diameter, compared with variation with the third power of helix length. Length values are normalised to 1 at zero distamycin concentration. Ethidium orientation angles were measured at 520 nm by extrapolation of the dichroism to infinite field as described in the text. a, (●), Length change for calf thymus DNA in absence of ethidium; b (○), angle change for calf thymus DNA in presence of ethidium; c, (×), length change for calf thymus DNA in presence of ethidium; d (△), length change of *E. coli* DNA without ethidium; e (□), length change of *E. coli* DNA with ethidium; f (■), angle change of *E. coli* DNA with ethidium present.

We also investigated the dichroism of ethidium at constant $r_{\text{distamycin}} = 0.03$ and varying r_{ethidium} values (Fig. 4). Up to about $r_{\text{ethidium}} = 0.1$, the dichroism extrapolated to infinite field was equal to -1.5 , but larger r_{ethidium} values led to a progressive decrease in the dichroism.

Finally, we examined the influence of bound distamycin ($r_{\text{distamycin}} = 0.03$) on the binding isotherm of ethidium, and

observed that ethidium binding becomes cooperative. We were not able to use the partition technique at the low salt concentrations used for Fig. 1, because the ethidium partition coefficient was concentration dependent in those conditions. Therefore, the data shown in Fig. 5 were measured at 1 M salt concentration. However, we verified that the distamycin-calf thymus DNA binding isotherm was cooperative in these salt conditions, with the distamycin binding affinity estimated from r/m at its peak reduced by about threefold from the value in Fig. 1. As seen in Fig. 5, r_{ethidium} values less than about 0.06 are characterised by lower r/m values than found in the absence of distamycin (open circles). Therefore, distamycin at $r_{\text{distamycin}} = 0.03$ substantially reduces the affinity of calf thymus DNA for ethidium, in the limit as r_{ethidium} approaches zero.

Interpretation as an allosteric transition of DNA

There are two general mechanisms which can lead to cooperative binding of drugs to nucleic acids, as found in Fig. 1; (1) the drugs can interact directly on the DNA lattice, as, for example, in the stacking of acridines on the DNA helix, or (2) the drug molecules can interact indirectly by altering the state of the nucleic acid. Examples of the latter are the ethidium-induced transformation of poly d(GC) from one form to another⁷, and conversion of poly dA·2poly rU from triple to double helix²². Mechanism (2) requires that the drug bind more tightly to the alternative form; for example, ethidium binds more tightly to double-helical poly dA·poly rU than to triple-helical poly dA·2poly rU. The process is analogous to allosteric conversion of a protein from a low to a high affinity form by an effector molecule.

Our results strongly support alternative (2), namely, that distamycin serves as an allosteric effector to convert calf thymus DNA from one form to another. The results with the ethidium probe are essential for this conclusion. The distamycin molecule is long enough to cover only about five DNA base pairs. Therefore, at $r_{\text{distamycin}} = 0.03$ only about 15% of the base pairs could be in contact with distamycin, leaving about 85% of the DNA molecule free. Thus, if the only effect were clustering of distamycin molecules, 85% of the DNA should show unaltered ethidium binding properties. However, the measured ethidium dichroism can reach the theoretical limiting value of -1.5 (Fig. 3) only if virtually all the ethidium molecules are bound perpendicular to the helix axis. Therefore, the 85% of the DNA that is distamycin-free cannot be binding ethidium in the same way as in the absence of distamycin, and we conclude that bound

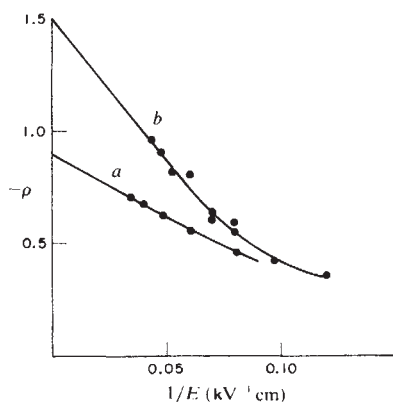


Fig. 3 Extrapolation of the observed dichroism of ethidium ($r_{\text{ethidium}} = 0.1$) to infinite field in the absence (a) and presence (b) of distamycin, $r_{\text{distamycin}} = 0.03$. Calf thymus DNA samples, dissolved in dichroism buffer, 11 °C.

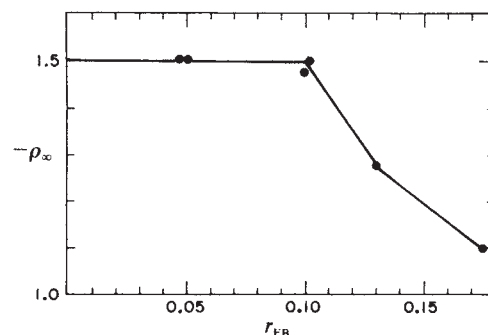


Fig. 4 Variation with extent of ethidium (EB) binding of the extrapolated dichroism of ethidium bound to calf thymus DNA in the presence of distamycin, $r_{\text{distamycin}} = 0.03$. Dichroism buffer, 11 °C.

distamycin alters the form of virtually all the DNA. This conclusion is also supported by the ethidium binding isotherm (Fig. 5), as distamycin at $r_{\text{distamycin}} = 0.03$ reduces the affinity for ethidium by at least a factor of two in the limit as r_{ethidium} approaches zero. This would not be possible if 85% of the DNA were unaltered in structure and ethidium affinity.

Comparison with theory

To determine quantitative features of the observed allosteric transition, we developed a general statistical mechanical theory of the process (manuscript in preparation). The model allows the DNA double helix to be in one of two forms: form (1) has a lower affinity for distamycin than form (2), but (1) has slightly lower free energy in the absence of distamycin and so is generally the

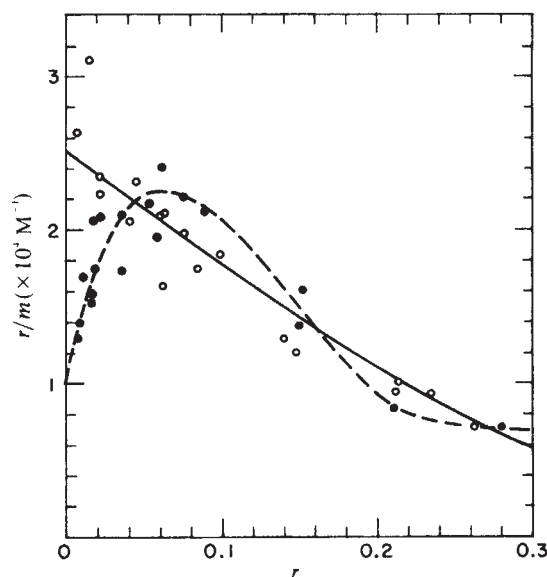


Fig. 5 Binding isotherm of ethidium to calf thymus in absence (O) and presence (●) of distamycin, $r_{\text{distamycin}} = 0.03$. r and m were measured by the phase partition technique as described in the legend to Fig. 1, except that samples were dissolved in PES buffer made up to a total concentration of 1 M in NaCl.

preferred structure. A positive interfacial free energy characterises each boundary between forms (1) and (2). Figure 1 shows a comparison of the experimental binding isotherm with a theoretical curve obtained by adjusting the parameters of the theory for best fit. The calculation reveals that the rising portion of the binding isotherm is associated with the cooperative conversion of the double helix from form (1) to (2). After the

maximum is reached, the transition is essentially complete and the curve follows the binding isotherm expected for pure form (2). The intrinsic distamycin binding constants⁵ are $0.3 \times 10^6 \text{ M}^{-1}$ for form (1) and $4.8 \times 10^6 \text{ M}^{-1}$ for form (2). A neighbour exclusion range⁵ of six was assumed for form (2) so as to fit the data beyond the maximum in r/m ; a more accurate theory would have to take account of the (unknown) base sequence preference of distamycin when binding to form (2) (ref. 5). The interfacial free energy necessary for the observed cooperative transition is $3\text{--}4 \text{ kcal mol}^{-1}$. Only a very small free energy difference, about $0.02 \text{ kcal per mol of base pairs}$, is found between forms (1) and (2). However, because of the cooperative character of the transition, less than 1% of form (2) is present in the absence of distamycin.

An important result of the calculation is that the average length of form (1) and form (2) helices is hundreds of base pairs in the transition region. This conclusion has two significant implications: first, allosteric effects in DNA can be transmitted over hundreds of base pairs, and, second the theory is only approximately applicable to the measurements, as the latter were done with DNA fragments about 150 base pairs long.

Characteristics of the altered form

Thus, three features of the DNA structure induced in calf thymus DNA by distamycin binding are (1) ethidium binds to form (2) with the long wavelength transition perpendicular to the DNA helix axis; (2) the apparent length of form (2) is substantially larger than that of form (1), by at least 13%, which

is the apparent length increase at the point where the cooperative transition to form (2) is essentially complete; (3) form (2) becomes distinctly shorter in apparent length as more distamycin is bound.

Why only calf thymus DNA?

Perhaps the most surprising feature of our results is the failure to obtain the effect with any DNA except that from calf thymus, out of three bacterial and two mammalian DNA tested. We believe that this is due to the requirement for near-equality of the free energies of the two forms, and the sensitivity of this free-energy balance to the details of base sequence or base modification, or both. If the average free energy difference of $20 \text{ cal per mol of base pairs}$ estimated between forms (1) and (2) in calf thymus DNA were increased to $200 \text{ cal per mol of base pairs}$ (a change per base pair considerably less than the thermal energy), the transition would not be observed. Hence, we conclude that the details of DNA base modification and sequence are extremely important for the long-range transmission of allosteric effects. Thus, negative results found in particular cases cannot be taken as general evidence against propagation of structural effects along DNA²³. The finding that distamycin acts as effector of an allosteric conversion in calf thymus DNA, when considered with previous evidence⁷⁻⁹, shows that DNA can transmit structural influences. It remains to be seen, however, whether such phenomena are of general importance in genetic regulation.

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1. Bourgeois, S. & Pfahl, M. *Adv. Protein Chem.* **30**, 1-99 (1976).
2. Hirsh, J. & Schleif, R. *Cell* **11**, 545-550 (1977).
3. Müller, W. & Crothers, D. M. *J. molec. Biol.* **35**, 251-290 (1968).
4. Bauer, W. & Vinograd, J. *J. molec. Biol.* **47**, 419-435 (1970).
5. Crothers, D. M. *Biopolymers* **6**, 575-584 (1968).
6. Sobell, H. M., Tsai, C. C., Jain, S. C. & Gilbert, S. G. *J. molec. Biol.* **114**, 333-365 (1977).
7. Pohl, F., Jovin, T. M., Baehr, W. & Holbrook, J. J. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3805-3809 (1972).
8. Burd, J. F., Wartell, R. M., Dodgson, J. B. & Wells, R. D. *J. biol. Chem.* **250**, 5109-5113 (1975).
9. Burd, J. F., Larson, J. E. & Wells, R. D. *J. biol. Chem.* **250**, 6002-6007 (1975).
10. Hahn, F. E. in *Antibiotics* Vol. 3 (eds Corcoran, J. W. & Hahn, F. E.) 79-100 (Springer, Berlin, 1975).
11. Luck, G. & Arcamone, F. *Nucleic Acids Res.* **4**, 2655-2670 (1977).
12. Wartell, R. M. & Wells, J. *biol. Chem.* **249**, 6719-6731 (1974).
13. Krey, A. K. & Hahn, F. E. *FEBS Lett.* **10**, 175-178 (1970).
14. Kolchinski, A. M. & Mirzabekhov, A. D. *Molec. Biol. (USSR)* **9**, 14-20 (1975).
15. Luck, G. & Zimmer, Ch. *Nucleic Acids Res.* **1**, 503-530 (1974).
16. Reinert, K. E. *J. molec. Biol.* **72**, 593-607 (1972).
17. Nosikov, V. & Jain, B. *Nucleic Acids Res.* **4**, 2263-2273 (1977).
18. Hogan, M., Dattagupta, N. & Crothers, D. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 195-199 (1978).
19. Hogan, M., Dattagupta, N. & Crothers, D. M. *Biochemistry* **18**, 280-288 (1979).
20. O'Konski, C. T., Yoshioka, K. & Orttung, W. H. *J. phys. Chem.* **63**, 1558-1565 (1959).
21. Allen, F. S. & Van Holde, K. E. *Biopolymers* **10**, 865-881 (1971).
22. Lehrman, E. A. & Crothers, D. M. *Nucleic Acids Res.* **4**, 1381-1392 (1977).
23. Selsing, E., Wells, R. D., Early, T. A. & Kearns, D. R. *Nature* **275**, 249-250 (1978).
24. Scatchard, G. *Ann. N.Y. Acad. Sci.* **51**, 660-672 (1949).
25. Eshaghpour, H. & Crothers, D. M. *Nucleic Acids Res.* **5**, 13-21 (1978).
26. Klevan, L. & Crothers, D. M. *Nucleic Acids Res.* **4**, 4077-4090 (1977).
27. Broersma, S. J. *chem. Phys.* **32**, 1626-1631 (1960).

Construction of a site-specific, deletion-frameshift mutation in an essential gene of bacteriophage ΦX174

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By in vitro methods, we have deleted 80 nucleotides from a preselected site in the coding sequence of gene G of bacteriophage ΦX174 . This mutant (G ∇ /FS ZH1) also carries a -2 frameshift. The mutation is lethal, but mutant virus can be stably maintained on host strains bearing plasmids carrying ΦX gene G.

To explore molecular mechanisms of mutation by carcinogens, we have developed a system for examining the *in vivo* expression of well characterised, site-specific, covalent modifications of biologically active DNA^{1,2}. Because of the extensive chemical and biological information available³⁻⁵, we have chosen an

essential gene (gene G) of the virulent phage, ΦX174 , for our initial studies. As the nature of the mutation produced by a site-specific modification cannot be predicted, we need a host that is permissive for all types of mutation in gene G. To deal with this problem in the most general way, we have developed cell lines that carry ΦX gene G on a plasmid¹. These p ΦXG -bearing hosts produce functional ΦX gene G protein and provide a means of rescuing ΦX174 carrying mutations in this gene.

We have demonstrated that cells carrying p ΦXG are permissive for conditionally lethal mutants (both nonsense and missense) in gene G (refs 1, 2 and R.W.C., unpublished results). A crucial test of our system is to show that p ΦXG -bearing host cells are also permissive for lethal mutations in gene G (frameshifts, large deletions and rearrangements).

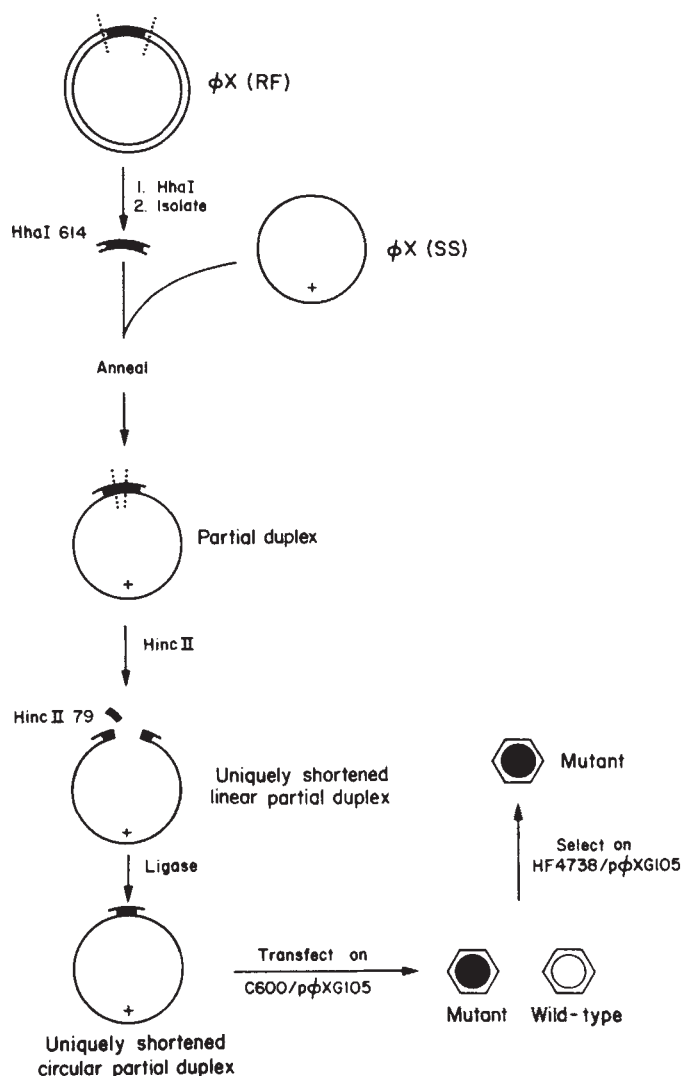


Fig. 1 Strategy for the construction of specific ΦX deletion mutants. The gene G coding sequence is indicated by the heavy black line. *HhaI* 614 (fragment H3) was isolated by procedures similar to those described elsewhere¹⁶. Wild-type ΦX viral DNA was prepared by phenol extraction of a purified phage preparation. Viral DNA (30 μ g) and *HhaI* (5 μ g) were annealed in 200 μ l of buffer (90 mM NaCl, 10 mM $MgCl_2$, 7.5 mM Tris-HCl, pH 8, and 0.75 mM EDTA) by boiling for 3 min, chilling rapidly to 0 °C and incubating at 67 °C for 2 h. The mixture (50 μ l) was made up to 100 μ l by adding buffers and enzymes to the following final concentrations: 45 mM NaCl; 12.5 mM Tris-HCl, pH 8; 5 mM 2-mercaptoethanol; 0.38 mM EDTA; 100 units ml^{-1} *HincII* (an isoschizomer of *HindII*; Bethesda Research Laboratories). Restriction was carried out for 2 h at 37 °C before termination by a phenol extract followed by three ether extractions and dialysis of the DNA against cold 10 mM Tris-HCl and 1 mM EDTA (pH 8). This DNA preparation was ligated at 20 °C for 5 h in 400 μ l with buffers and enzyme at the following concentrations: 50 mM Tris-HCl, pH 7.5; 5 mM $MgCl_2$; 5 mM 2-mercaptoethanol; 0.08 mM ATP; 0.25 mM EDTA; 50 units ml^{-1} T4-DNA ligase. The reaction was terminated by one phenol and three ether extractions. The DNA was redialysed against cold 10 mM Tris-HCl and 1 mM EDTA (pH 8). Spheroplasts were prepared as described elsewhere¹⁷ from C600/p $\Phi XG105$ constructed by transforming the K12 strain C600 (Su^- , ΦX^+) with p $\Phi XG105$ DNA by methods described elsewhere¹. The above ligated DNA (40 μ l) solution was diluted to 400 μ l with 50 mM Tris-HCl (pH 8) and used to transfect 400 μ l of the spheroplast preparation by published procedures¹⁷. Spheroplast lysates plated on the *E. coli* C/K12 strain, HF4738 ΦX^+ Su^+ *recA*/p $\Phi XG105$, gave about 10^7 plaque-forming units per μ l of the ligated DNA preparation. The lysate was screened for lethal mutants by plating on double-layer lawns of HF4738/p $\Phi XG105$ over HF4738. After 7 h at 37 °C the plates revealed that 2–5% of the plaques were small and turbid, and the remaining plaques had wild-type morphology (large and clear). Thirty turbid plaques were punched out at random with a capillary. Each plaque was suspended in buffer (50 mM sodium borate, 1 mM EDTA, pH 9). Each suspension was titrated at suitable dilutions on C, HF4738 and HF4738/p $\Phi XG105$, and nine were found to plate on C and HF4738 at a plating efficiency lower than 10^{-4} compared with that on HF4738/p $\Phi XG105$. One of these suspensions constituted the seed stock for ΦX *G ∇ /FS ZH1*.

Here, we report the *in vitro* construction of a site-specific deletion-frameshift mutant, *G ∇ /FS ZH1*, in which residues 2,513–2,592 (80 bases) of the wild-type sequence have been deleted. This also produces a –2 frameshift in which 14 in-frame, nonsense codons are generated at, and downstream from, the site of the deletion. We will show that this mutation is lethal, but the mutant virus grows in host cells carrying p ΦXG . The procedure we have used for constructing this deletion frameshift is simple and specific. It should be applicable not only to other regions of $\Phi X174$ DNA but also to other interesting DNAs, provided that a system for growing the mutant is available. The results also demonstrate that it should be possible to rescue most $\Phi X174$ gene G mutants regardless of the origin or nature of the mutation.

Construction of $\Phi X174$ *G ∇ /FS ZH1*

Figure 1 is a scheme of the procedures used for the construction and isolation of the deletion mutant. This involved the following steps. (1) ΦX wild-type replicative form DNA (RF DNA) was cleaved with the restriction endonuclease *HhaI*, and the fragment *HhaI* 614 (H3)³, was isolated. This fragment contains all the translated portion of gene G, 29 of the nucleotides preceding the translated portion of gene H and 49 residues from gene H (ref. 3). (2) Wild-type ΦX single-stranded viral DNA (SS DNA) was annealed with double-stranded restriction fragment *HhaI* 614. (3) The ΦX SS DNA–*HhaI* 614 partial duplex was cleaved with *HincII* (an isoschizomer of *HindII*) at the known *HindII* sites, R9/10 and R10/2 (ref. 3). These cut sites are 79 nucleotides apart and include residue 2,571, the preselected target for our projected modification studies. (4) Without isolation, the specifically shortened, linear, partial duplex was rejoined by blunt-end ligation with T4 DNA ligase to obtain a covalently closed, circular, partial duplex with a unique deletion. (5) Infectious virus particles were produced by transfection of C600/p $\Phi XG105$ spheroplasts with the mixture containing uniquely shortened circular partial duplex DNA. (6) Mutants were selected from the resulting phage population by plating on double-layer lawns of HF4738/p $\Phi XG105$ over HF4738.

In a typical experiment (see legend to Fig. 1), 2% of the phage population obtained after step (5) were mutant by the criterion of plaque formation on HF4738/p $\Phi XG105$ but not on the parent strain HF4738. Three such mutants were randomly selected for further analysis and were found to have similar properties. Most of the experiments described below refer to one of these mutants, named *G ∇ /FS ZH1*.

Biological characterisation of *G ∇ /FS ZH1*

Table 1 lists some of the ΦX -sensitive host strains that have been tested for plaque formation with *ZH1*. (The abbreviation *G ∇ /FS ZH1* means a deletion and frameshift mutation in gene G, isolate *ZH1*. For simplicity, we will mainly use the shortened designation *ZH1*.) The data show that the mutant will not grow on host strains without p $\Phi XG105$. These experiments demonstrate that the genetic defect in *ZH1* is lethal, and is confined to gene G, as p $\Phi XG105$ is a well characterised plasmid known to complement for gene G product(s) alone (refs 1, 2; M.Z.H. and R.W.C., unpublished). Also, in a complementation experiment carried out by methods similar to those described by Zuccarelli *et al.*⁶, the gene H *amber* mutant, *N1* (ref. 7), but not the gene G mutant, *am9* (ref. 5) is rescued by *ZH1*. No infective particles are observed in artificial (freeze-thaw) lysates of the wild-type host, *Escherichia coli* C, infected with *ZH1*. Thus, a defect in lysis cannot be solely responsible for the lethality of the mutation. *ZH1* does not grow on *E. coli* C at either 30 or 42 °C.

Single-cell, single-burst experiments, as well as one-step growth experiments⁸, revealed that *ZH1* has a burst size of about 20 on HF4738/p $\Phi XG105$. Wild-type ΦX has a burst size of 200–300 on a similar strain with or without p $\Phi XG105$ (M.Z.H., unpublished results). We have recently shown that the small burst size of *Gam9* on p ΦXG -bearing strains is attributable to its known polar effect on the expression of gene H (M.Z.H., unpublished results). The low burst size of *ZH1* is

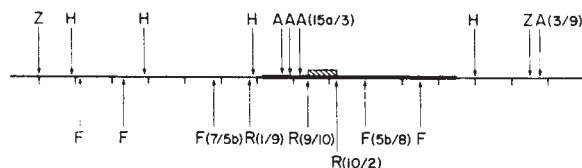


Fig. 2 A schematic representation of a part of the physical map of ΦX wild-type RF DNA to show the site of the deletion in *ZH1* (hatched rectangle). Each unit is approximately 100 base pairs. The cutting sites of some relevant restriction enzymes are indicated by vertical arrows identified by the letters A (*AluI*), F (*HinfI*), H (*HhaI*), R (*HindIII*) and Z (*HaeIII*). Fragments separated by cleavage at some of these sites³ are shown in parentheses. The coding sequence for gene G principal product is indicated by a thick line.

probably a result of nonsense codons generated downstream from the deletion.

Recombination occurs between *ZH1* and $p\Phi XG105$, although it is quantitatively less than recombination between *Gam9* and $p\Phi XG105$ (ref. 1). The level of recombination is further reduced by at least two orders of magnitude in a *recA*⁻ background (such as HF4738) as compared to a *recA*⁺ background (H514). For example, in one experiment single plaques produced by *ZH1* on plasmid-bearing *recA*⁺ strains, picked after incubation for 5–8 h at 37 °C, showed 1–5% wild-type recombinants, whereas similar plaques formed by *Gam9* showed at least 50% wild type. On a *recA*⁻ lawn, *Gam9* and *ZH1* gave 10^{-4} and 10^{-5} wild-type levels, respectively, in plaques picked after incubation for about 5 or 6 h at 37 °C.

These data show that *ZH1* is a gene G mutant; it is not temperature sensitive, and does not grow on any of the nonsense suppressor strains tested (Table 1). It can be maintained in a stable, apparently normal, virulent cycle on host strains carrying $p\Phi XG$, implying that it is capable of all phase functions except those performed by the gene G product(s). It has a low burst size on $p\Phi XG$ -bearing strains, and its recombination frequency with $p\Phi XG$ is lower than that of the point mutant, *Gam9*, with $p\Phi XG$.

Characterisation of *G^v/FS ZH1* DNA

ZH1 RF, as well as SS DNA preparations, have electrophoretic mobilities very close to those exhibited by the corresponding

wild-type DNAs on 1% agarose gels. The predicted deletion (approximately 1.5% of the ΦX genome) is not expected to change the mobility of *ZH1* DNA appreciably compared with wild type in these conditions.

The known restriction sites in gene G of $\Phi X174$ RF DNA and the method of constructing *ZH1*, enable us to draw a new (expected) cleavage map for *ZH1* (Fig. 2). This prediction was examined with five restriction enzymes.

Analysis of cleavage products from *ZH1* RF DNA produced by four restriction endonucleases (*HaeIII*, *HhaI*, *AluI* and *HinfI*; Fig. 3) revealed that 80 ± 3 base pairs have been deleted from wild-type DNA, as expected. Digestion of *ZH1* with *HincII* revealed not only the loss of fragment R10 (79 base pairs), as expected, but also a fusion between fragment R9 (162 base pairs) and fragment R2 (770 base pairs) that flank fragment 10 in the wild type⁹. This generates a new fragment about 930 base pairs long. Our construction strategy was expected to regenerate a single *HincII* site by fusing the left half of the site separating R9 and R10 and the right half of the site separating R10 and R2 in the wild-type DNA⁹. Our results with *HincII* (Fig. 3) show that this expected site was not formed. All the restriction fragments seem to be sharp and, with the exceptions noted above, identical to the corresponding wild-type fragments (Fig. 3). This suggests that except for the deletion, the wild-type sequence has been conserved in *ZH1* to the extent definable by restriction.

To characterise the mutation precisely, we determined the DNA sequence of *ZH1* around the expected site of the deletion as well as around other selected parts of the gene G coding sequence³. The results (Figs 4, 5) unambiguously show the loss of an 80 nucleotide-long sequence extending from base number 2,513 to 2,592 of the revised sequence by Sanger *et al.*⁹. Of these deleted 80 nucleotides, 79 are present in the *HincII* fragment 10. The loss of a single, additional base pair (CG) at position 2,513 eliminates the *HincII* site that would otherwise be regenerated by ligation (Fig. 1). Although we cannot rule out an *in vivo* event for the loss of this base pair, we believe that *ZH1* has been derived from a parent molecule in which this additional loss occurred during *in vitro* manipulations. Some of our original mutant isolates may have the expected deletion of only 79 nucleotides. Except for the deletion, we observe no deviation in the *ZH1* sequence so far determined, compared with the wild-type sequence in gene G (ref. 9).

Fig. 3 Fractionation of restriction endonuclease products from ΦX *G^v/FS ZH1* RF DNA by polyacrylamide gel electrophoresis. The DNA was prepared by infecting *E. coli* C grown in 3XD to 5×10^8 cells ml^{-1} with ΦX *G^v/FS ZH1* ($<10^{-4}$ wild type) at a multiplicity of infection of 3. Ten min after infection, chloramphenicol was added to a concentration of $30 \mu g\ ml^{-1}$ and shaking was continued for 3 h at 37 °C. Lysis, removal of host DNA and ethanol precipitation were carried out exactly as described by Godson and Vapnek¹⁸. The DNA pellet from a 1-l culture after ethanol precipitation was suspended in 20 ml 50 mM Tris-HCl:5 mM EDTA (pH 8). The turbid suspension was extracted three times with phenol using centrifugation to separate the phases after each extraction. The aqueous layer was extracted three times with ether and the solution dialysed against the above Tris-EDTA buffer. The solution was adjusted to pH 12.11 at 25 °C with NaOH. The pH and ionic strength at this step are critical¹⁹. The pH was measured with a radiometer type B glass electrode with a negligible Na⁺ correction in these conditions. The electrodes were standardised with fresh $Ca(OH)_2$ just before use. The pH was maintained for 10 min at 25 °C before neutralising the solution with HCl. Pancreatic RNase A ($20 \mu g\ ml^{-1}$; heat treated at 70 °C for 15 min) was added and the solution was incubated at 37 °C for 30 min before concentrating *in vacuo* to ~2 ml and fractionating the DNA on a Sephracryl S200 column (2.5×35 cm), equilibrating and eluting with 0.3 M NaCl and 0.03 M sodium citrate, pH 7 (Sephadex G-100 gives similar results). Fractions containing the RF DNA eluted near the void volume as a discrete peak. These fractions were pooled and dialysed extensively against cold 10 mM Tris-HCl and 1 mM EDTA (pH 8). The average yield of this preparation is about 1 mg of RF DNA per l of culture. About 2 μg of *ZH1* or wild-type RF DNA were digested in 100 μl with *HaeIII*, *HhaI*, *AluI* or *HinfI* and the products were fractionated on 5% polyacrylamide slab gels¹⁶. The *HincII* digest was end-labelled with ^{32}P as described elsewhere¹⁶ and fractionated on 10% (a) and 5% (b) polyacrylamide gels. The wild-type fragments affected by the deletion are indicated by solid arrows; the fragment resulting from the deletion is shown by a broken arrow. The approximate size in base pairs is indicated and is based on electrophoretic mobility relative to the known wild-type fragments.

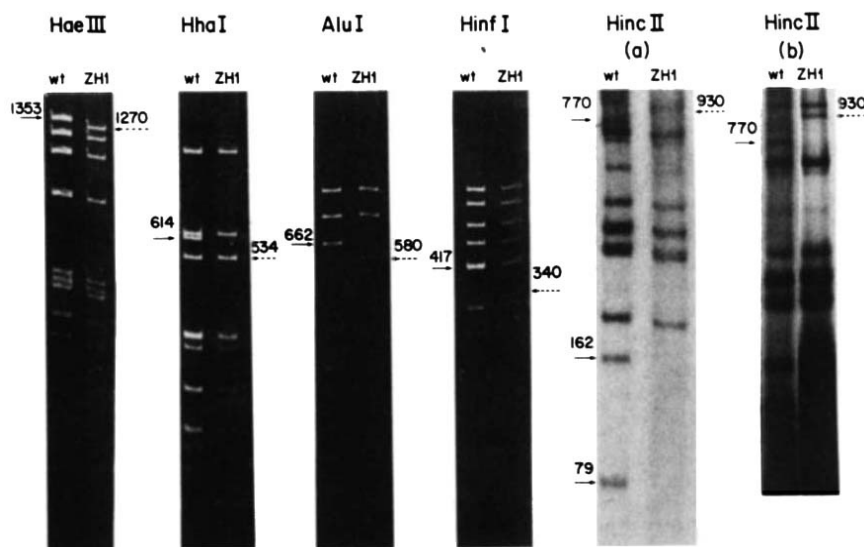


Table 1 Permissivity of Φ X174 ZH1 on various host strains

Host strain and relevant characteristics	Plaque formation by Φ X174 ZH1	Efficiency of plating relative to HF4738/p Φ XG105
C (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
H514 (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
HF4704 (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
HF4714 (rec ⁺ , Su ⁻) [*]	-	<10 ⁻⁴
HF4738 (rec ⁺ , Su ⁻) [*]	-	<10 ⁻⁴
HF4740 (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
C1757 (rec ⁺ , Su ⁻) [*]	-	<10 ⁻⁴
C1792 (rec ⁺ , Su ⁻) [*]	-	<10 ⁻⁴
CR63.1 (rec ⁺ , Su ⁻) [*]	-	<10 ⁻⁴
SuVIII (rec ⁺ , Su ⁻) [†]	-	<10 ⁻⁴
CIT103 (rec ⁺ , Su ⁻) [‡]	-	<10 ⁻⁴
H514/pMB9	-	<10 ⁻⁴
H514/p Φ XG105	+	0.9
HF4704/p Φ XG105	+	0.8
HF4738/p Φ XG105	+	1.0
HF4740/p Φ XG105	+	1.2
C1757/p Φ XG105	+	0.6
C1792/p Φ XG105	+	0.4
C600 (Φ X ⁻ , Su ⁻) spheroplasts [§]	-	
C600 (Φ X, Su ⁻) spheroplasts [§]	-	
C600 Su ⁻ /p Φ XG105 spheroplasts [§]	+	

^{*} amber (UAG) suppressor; [†] amber (UAG) and ochre (UAA) suppressor; [‡] opal (UGA) suppressor; [§] these Φ X⁻ strains were transfected with viral DNA.

Some consequences of the mutation in *GV/FS ZH1*

Examination of the expected (and partially confirmed) sequence of gene G from *ZH1* (Fig. 5) reveals that the deletion eliminates 27 out of 175 gene G codons. However, a total of 136 gene G codons are affected because of a -2 shift in the reading frame that occurs at the site of the deletion. Thus, the gene G sequence in *ZH1* can code for a polypeptide with a sequence identical to the first 39 amino acids from the amino terminus of the wild-type, gene G spike protein. The deletion creates a -2 frameshift in the 40th codon (residue 2,512, Fig. 5) converting a serine codon (UCA) to an ochre codon (UAA). (All the Φ X mRNAs are transcribed from the minus strand of Φ X RF DNA. Therefore, they have the same sense as the viral strand⁴.) Presumably, this leads to premature termination of translation. Downstream from the site of the deletion, there are 13 additional, in-frame, nonsense codons (7 UAA and 6 UGA), and several codons that might allow reinitiation, but translation from any of these possible sites encounters in-frame nonsense codons. Because of this nonsense codon barrier, it seems unlikely that any gene G product large enough to have gene G spike protein function will be coded from *ZH1* DNA. This would explain why this particular mutation is lethal and why complementation is required to produce infectious virus particles.

The generation of nonsense codons also seems to lead to abnormal termination of transcription. Our main evidence for this is the small burst size (~20) of *ZH1* grown in a p Φ XG105-bearing strain compared with that of wild-type Φ X (~300) on the same strain. We believe this is a result of the nonsense codons that are generated downstream from the mutation in *ZH1* and the polar effect of these nonsense codons on the adjacent gene, gene H.

Note that gene G and H products are both essential proteins^{4,5}. Both products are made from polycistronic mRNA(s)¹⁰⁻¹⁴. Cells bearing p Φ XG105 make large amounts of Φ X gene G protein even in the absence of viral infection (M.Z.H., unpublished results). The mutant virus must provide all other required proteins, including gene H product, for an infectious particle to be produced. With certain polar Φ X mutants, such as *Gam9*, production of gene H product is markedly reduced¹⁵. We have shown that this polar effect persists with *Gam9* grown in p Φ XG105-bearing cells, giving

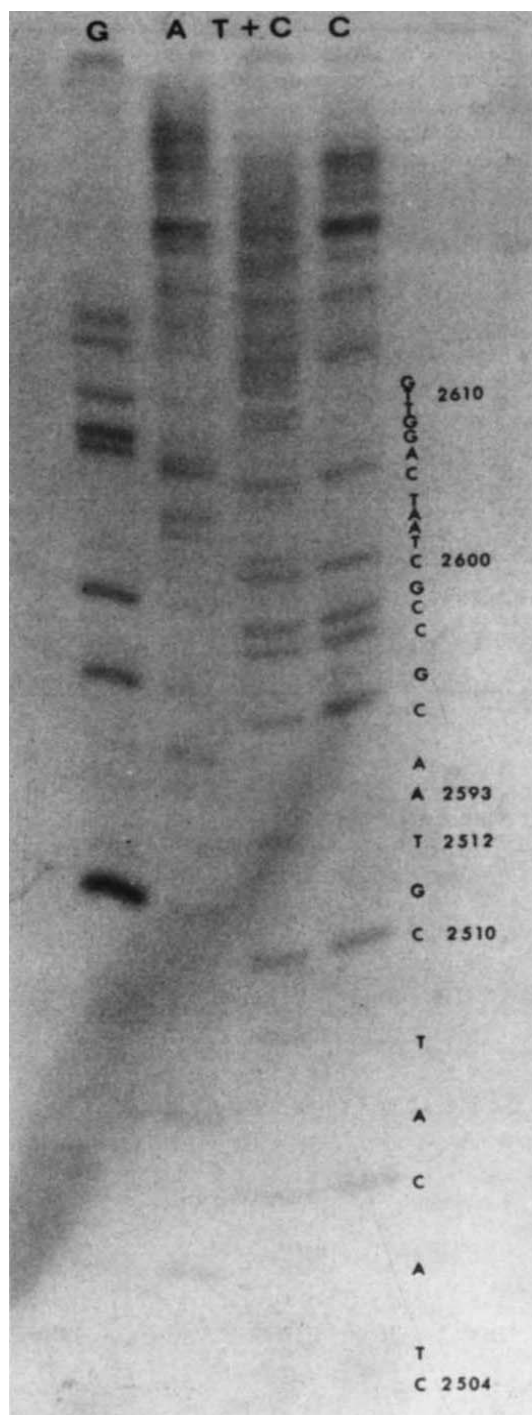


Fig. 4 Autoradiograph of a Maxam-Gilbert²⁰ sequence gel. For this sequence, the *ZH1* *AluI* fragment 3 (Fig. 3) was isolated, end-labelled with ³²P and redigested with *HaeIII* to generate *AluI/HaeIII* ~546 labelled at the *AluI* end by the methods described by Humayun *et al.*¹⁶. Partial chemical degradation products were fractionated on a 25% polyacrylamide-7 M urea gel. Nucleotides are numbered according to Sanger *et al.*⁹. Note the deletion of the wild-type sequence from 2,513 to 2,592. The ambiguity in the first two residues at the 5' end of A3 (bottom of figure) is resolved by the known specificity of *AluI*, AG/CT. Thus, the first two residues must be CT, in agreement with the published sequence.

rise to a small burst size (10–15) that is increased five- to seven-fold by an *amber* suppressor strain (non-permissive by itself) carrying pΦXG105.

The generation of 14 nonsense codons in *ZH1* brings this mutant close to a possible 'worst-case' mutation elicited by a site-specific modification of DNA in this region. Before the isolation of *ZH1*, we considered the possibility that the generation of a large number of nonsense codons would be so polar for gene H that the mutant would not be rescued by pΦXG. Our results indicate that polar effects do not seem to affect our studies as seriously as we had anticipated.

Concluding remarks

The isolation and characterisation of ΦX174 *G*∇/*FS* *ZH1*, shown here to be a deletion-frameshift mutant at a preselected site in gene G, an essential gene of ΦX174, validates the biological part of the system developed in this laboratory for studying the molecular mechanisms of mutation by carcinogens. We have now shown that nonsense mutations^{1,2}, missense mutations (R.W.C., unpublished results), frameshift mutations (this work) and 'large' deletion mutations (this work) can be isolated using host cells carrying pΦXG. Thus, it should be

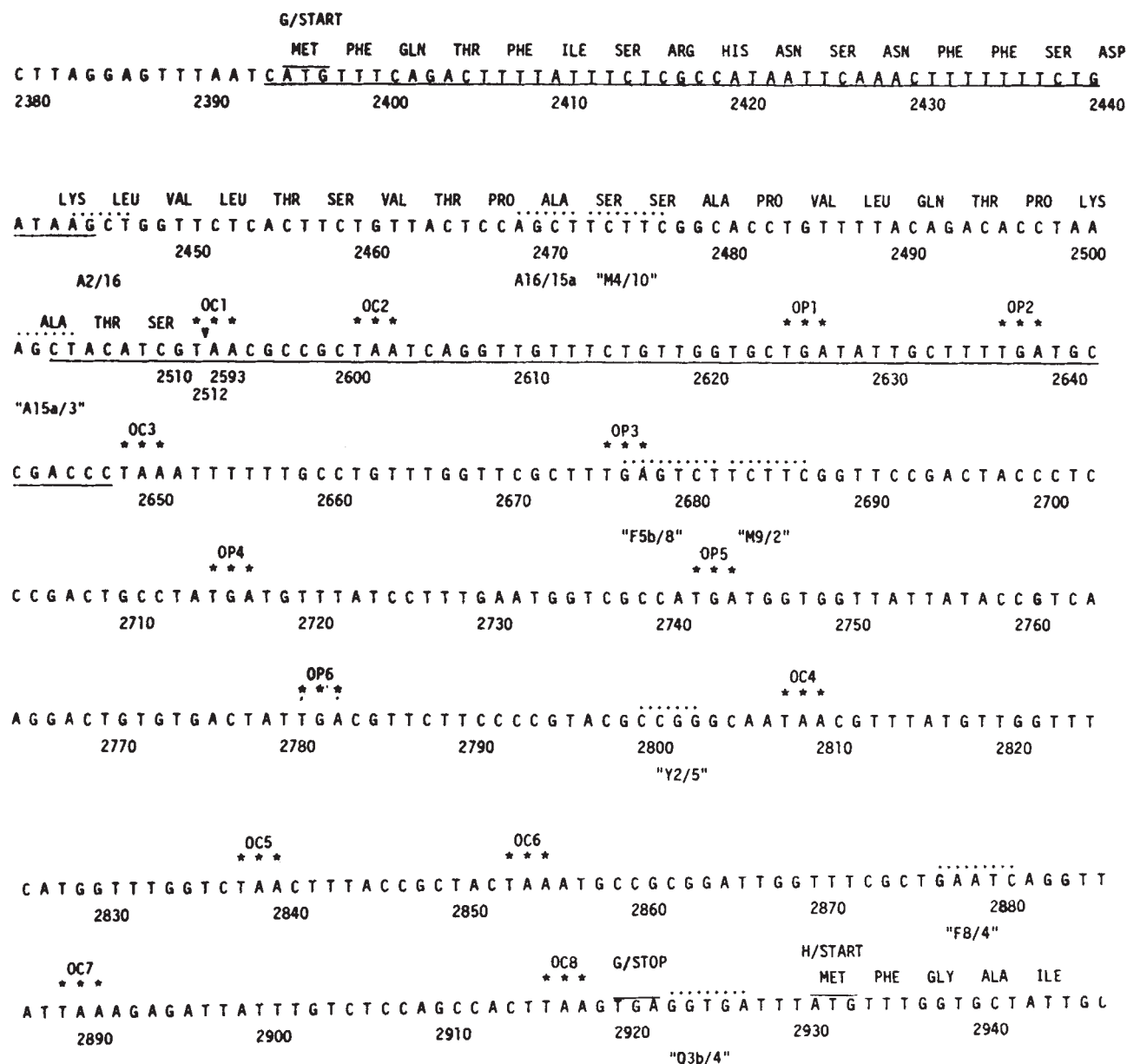


Fig. 5 Predicted (and partially confirmed) nucleotide sequence of gene G in *ZH1*. The sequence indicated by a continuous line was determined by us by the Maxam-Gilbert²⁰ technique. The remaining sequence and the nucleotide numbering were those of Sanger *et al.*⁹ In the nucleotide sequence, the sites of some restriction endonucleases are indicated by dotted overlining and are identified by the nomenclature used by Sanger *et al.*³ (A = *AluI*; F = *HinfI*; M = *MboII*; Q = *HphI*; R = *HindII* and Y = *HpaII*). For ease of comparison, we have also retained the cutting site identifications (fragments separated by each site) of Sanger *et al.*³, but note that the deletion in *ZH1* affects the sizes of some of the fragments produced by restriction. The site of the deletion and frameshift is indicated by an inverted solid triangle. The wild-type translational start signals for gene G (G/START at position 2,395) and gene H (H/START at position 2,931), as well as the wild-type G translational stop signal (G/STOP at position 2,920), which is now out of phase with the G start signal, are identified by solid overlining. Nonsense codons (for example, 8 UAA; OC1, 6 UGA; OP1 and so on) encountered in the same reading frame as the G start signal are identified by asterisks. The sequence of a 39 amino acid-long polypeptide possibly coded by the mutated gene G sequence in *ZH1* (identical to the first 39 amino acids from the N-terminus of the wild-type G product) is indicated.

possible to isolate any mutant that might be produced by a single, preselected, site-specific, covalent modification of ΦX gene G with a given carcinogen. The nature of the mutation(s) produced by the premutational lesion in the parental DNA can then be determined by sequencing the mutant, progeny DNA.

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- Humayun, M. Z. & Chambers, R. W. *Proc. natn. Acad. Sci. U.S.A.* **75**, 774–778 (1978).
- Bhanot, O. S. & Chambers, R. W. *J. biol. Chem.* (submitted).
- Sanger, F. *et al. Nature* **265**, 687–695 (1977).
- Denhardt, D. T. in *Comprehensive Virology* Vol. 7 (eds Fraenkel-Conrat, H. & Wagner, R. R.) 1–104 (Plenum, New York, 1977).
- Sinsheimer, R. L. in *Progress in Nucleic Acid Research and Molecular Biology* Vol. 8 (eds Davidson, J. N. & Cohn, W. E.) 115–169 (Academic, New York, 1968).
- Zuccarelli, A. J., Benbow, R. M. & Sinsheimer, R. L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1905–1910 (1972).
- Hayashi, M. N. & Hayashi, M. *J. Virol.* **14**, 1142–1151 (1974).
- Adams, M. H. *Bacteriophages* (Interscience, New York, 1959).
- Sanger, F. *et al. J. molec. Biol.* **125**, 227–248 (1978).

- Hayashi, M. N. & Hayashi, M. *Cold Spring Harb. Symp. quant. Biol.* **35**, 171–174 (1970).
- Sedat, J. W. & Sinsheimer, R. L. *Cold Springs Harb. Symp. quant. Biol.* **35**, 165–170 (1970).
- Clements, J. B. & Sinsheimer, R. L. *J. Virol.* **15**, 151–160 (1975).
- Hayashi, M., Fujimura, F. K. & Hayashi, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3519–3523 (1976).
- Axelrod, N. *J. molec. Biol.* **108**, 753–779 (1976).
- Benbow, R. M., Mayol, R. F., Picchi, J. C. & Sinsheimer, R. L. *J. Virol.* **10**, 99–114 (1972).
- Humayun, M. Z., Jeffrey, A. & Ptashne, M. *J. molec. Biol.* **112**, 265–277 (1977).
- Guthrie, G. D. & Sinsheimer, R. L. *J. molec. Biol.* **2**, 297–305 (1960).
- Godson, G. N. & Vapnek, D. *Biochim. biophys. Acta* **299**, 516–520 (1973).
- Pouwels, P. H., Knijnenburg, C. M., van Rotterdam, J., Cohen, J. A. & Jansz, H. S. *J. molec. Biol.* **32**, 169–182 (1968).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).

letters

A search for X-ray pulsations from the Galactic Centre

AN intriguing problem in astrophysics is the determination of the conditions which prevail at the centre of the Galaxy. X-rays can penetrate the intervening dust and gas which obscure visible light from this region, and we show here that they may thus provide a sensitive probe of the hot gas and peculiar objects which may populate the Galactic Centre.

X-ray emission was first detected from an extended ($\sim 2^\circ$) region in the direction of the Galactic Centre by Kellogg *et al.*¹. With the spatial resolution of the Uhuru detector used for that observation, it was not possible to determine whether the emission was diffuse, or whether discrete sources contributed to at least part of the flux from this region (designated GCX). Discrete, albeit short-lived sources were later discovered there—two transients^{2,3} and at least three bursters⁴. Lewin *et al.*⁴ noted that two of the three burst sources might be the same as the above transients. They also pointed out that many burst sources have a 'steady' X-ray component which has a time-averaged luminosity ~ 100 times that of the bursts. It was therefore proposed that the GCX bursters also have steady components which would provide a major part, or perhaps even all of GCX flux. This idea was supported by the subsequent detection of several point sources in GCX^{5,6}, at which time it was also noted that the positions of some of the steady sources were in agreement with the positions of the bursters and transients.

The sky distribution of burst sources⁷ and the absence of pulsations, periodicities, and eclipses in the steady components of many other burst sources⁸ make it plausible that the bursters are Pop. II objects, similar to the 'galactic bulge' and the X-ray emitters in globular clusters⁹. However, there may be other sources in the GCX complex which are not associated with bursters. Some of these might be Pop. I objects which might produce pulsations. Radio observations testify to Pop. I activity near the Galactic Centre: that is, active regions of star formation and the presence of supergiant stars and ionising O and B stars. Therefore, it is not impossible that transient X-ray sources such

as the Be stars A1118–61 and A0535+26, which showed pulsations of 405 s and 104 s respectively^{10,11}, might also be detected at the centre of the Galaxy.

We aimed to determine the nature of the discrete sources in GCX by looking for periodic X-ray behaviour. To do this, we used data taken from long pointing observations of GCX (6.3 h in 1976 Feb and 18.9 h in 1977 Feb) by the SAS 3 X-ray observatory. We used the horizontal tube system¹² which had a 1.7° (FWHM) field of view and spanned the energy interval 2.5–35 keV. The data were analysed using the Cooley–Tukey fast Fourier transform (FFT) algorithm. A complete description of the data reduction techniques is given elsewhere¹³. The sampling length used in this analysis restricted our search to periods > 1.7 s, while the low-frequency noise introduced by spacecraft pointing jitter made the search meaningful only for periods ≤ 40 min.

No periodic X-ray behaviour was detected from GCX. In the energy range 2.5–10 keV the 90% confidence upper limits to pulsations range from $\sim 1.6 \times 10^{-3}$ ct cm⁻² s⁻¹ for periods from 2 to 20 s, to $\sim 1.0 \times 10^{-2}$ ct cm⁻² s⁻¹ for periods of the order of 30 min. These correspond to pulsed fractions of the total GCX flux of 1.1% and 7%, respectively. In the 8–35 keV band these limits range from $\sim 2.3 \times 10^{-3}$ ct cm⁻² s⁻¹ (3.3% of the total GCX flux) for periods $\leq 6 \times 10^3$ s, to 6.7×10^{-3} ct cm⁻² s⁻¹ (9.5% of GCX) for longer periods up to 40 min. (The calculation of the pulsed fractions applies only for a sinusoidal pulsation.)

If we assume, as seems likely, that part of the steady GCX flux is due to the steady contribution from bursters, we note the similarity between these and the other X-ray bursters. For the latter, Cominsky *et al.*⁸ have determined upper limits to the X-ray pulsed fraction of a few per cent (2–66 s) and 10% (66–1,000 s). Therefore, some of the discrete GCX sources may be similar to the other bursters which have been associated with Pop. II type X-ray sources. As noted by Lewin (ref. 14 and refs. therein) the absence of pulsations is not compelling evidence against the proposed binary-neutron star models for the burst sources. The magnetic fields of these objects may have decayed (ref. 15 and refs. therein) or, alternatively, the neutron stars in these systems may never have had strong magnetic fields.

In conclusion, we have not detected any pulsations in the range $\sim 2\text{--}2,000\text{ s}$ from GCX, a region which is known to contain several point sources. However, a search for periodicities $<2\text{ s}$ and $>30\text{ min}$ would be valuable.

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1. Kellogg, E., Gursky, H., Murray, S., Tannanbaum, H. & Giacconi, R. *Astrophys. J. Lett.* **169**, L99 (1971).
2. Eyles, C. J., Skinner, G. K., Willmore, A. P. & Rosenberg, F. D. *Nature* **257**, 291 (1975).
3. Ariel V Group, Univ. Birmingham. *IAU Circ. No.* 2934 (1976).
4. Lewin, W. H. G. *et al. Mon. Not. R. astr. Soc.* **177**, 83 (1976a).
5. Cruddace, R. G., Fritz, G., Shulman, S. & Friedman, H. *Astrophys. J. Lett.* **222**, L95 (1978).
6. Proctor, R. J., Skinner, G. K. & Willmore, A. P. *Mon. Not. R. astr. Soc.* **185**, 745 (1978).
7. Lewin, W. H. G. *et al. Nature* **267**, 28 (1977).
8. Cominsky, L., van Paradijs, J. & Lewin, W. H. G. (in preparation).
9. Li, F. K., Clark, G. & Markert, T. *Nature* **275**, 723 (1978).
10. Ives, J. C., Sanford, P. W. & Bell-Burnell, S. J. *Nature* **254**, 578 (1975).
11. Rosenberg, F. D., Eyles, C. J., Skinner, G. K. & Willmore, A. P. *Nature* **256**, 628 (1975).
12. Lewin, W. H. G. *et al. Mon. Not. R. astr. Soc.* **177**, 93 (1976b).
13. Córdova, F., Garmire, G. & Lewin, W. H. G. *Final Tech. Rep.* (NASA Grant NSG 5197, 1978).
14. Lewin, W. H. G. *Proc. COSPAR Symp.*, Innsbruck (1978).
15. Flowers, E. & Ruderman, M. *Astrophys. J.* **215**, 302 (1977).

The angular size of the high-redshift quasar Q0000–398

THE optically selected quasar¹ Q0000–398 has a redshift² $z = 2.827$. This quasar has been identified with a faint double radio source whose angular extent is $134 \pm 40\text{ arc s}$. As all known high-redshift radio-selected quasars have much smaller angular sizes, it has been suggested that the upper envelope of the angular size–redshift diagram of radio quasars is depressed by an anticorrelation of linear size and radio luminosity³. We show here, first that the radio source associated with Q0000–398 is compact and centred on the optical quasar, and second that there is no evidence in our data on other quasars for the suggested anticorrelation.

We observed Q0000–398 at 1,465 MHz with the partially completed VLA during 16–18 June 1978 as part of a larger programme to study radio emission from optically selected quasars. Nine scans of Q0000–398 were made at uniform intervals within 2 h of transit. All 15 pairs of the six antennas were correlated; baselines from 3,500 to 52,000 wavelengths were available. The synthesised beam is shown in Fig. 1a. The 'clean' map of Q0000–398, restored with an 18 by 3 arc s elliptical gaussian beam, is shown in Fig. 1b.

The only radio source in the field is at 1950.0 position $\alpha = 00^{\text{h}}00^{\text{m}}30.29 \pm 0.02$, $\delta = -39^{\circ}48'47.6 \pm 0.8$. It is unresolved (<6 by 1 arc s) and has a 1,465 MHz flux density of $14 \pm 2\text{ mJy}$. The optical position of Q0000–398, measured from the Whiteoak extension of the National Geographic–Palomar Observatory Sky Survey print, is $\alpha = 00^{\text{h}}00^{\text{m}}30.26 \pm 0.13$, $\delta = -39^{\circ}48'49.6 \pm 1.5$. Thus we identify Q0000–398 with a single compact radio source centred on the optical quasar. M. R.

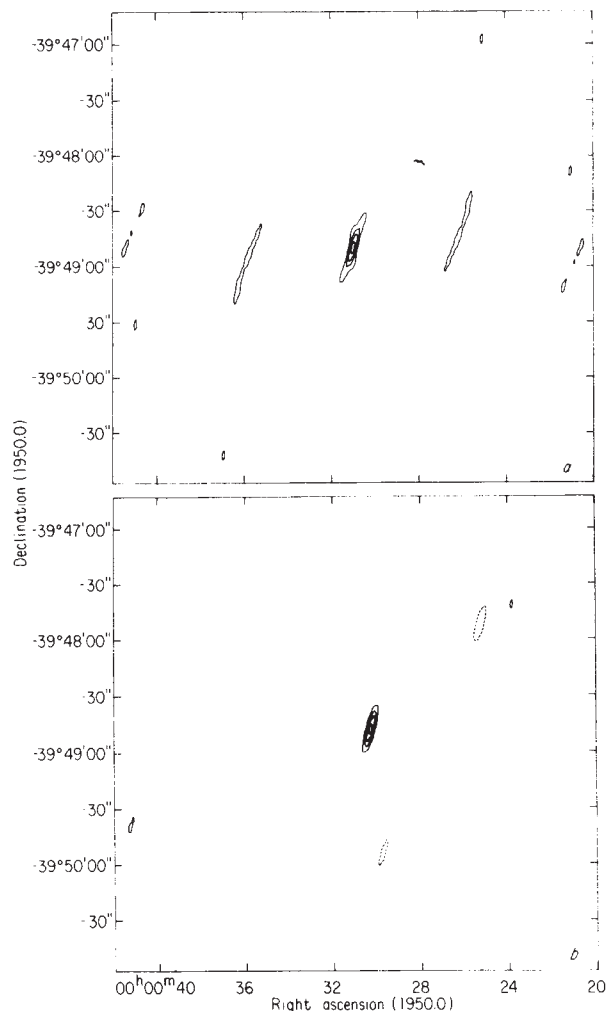


Fig. 1 a, Synthesised beam for the 1,465 MHz VLA observations of Q0000–398. b, The 1,465 MHz VLA map of Q0000–398. Negative contours are indicated by broken lines.

Gearhart (personal communication) has suggested that marginal observing weather coupled with the low elevation of the source may be responsible for the large angular size observed at 2,695 MHz with the NRAO three-element interferometer.

Our preliminary 1,465 MHz VLA maps of other high-redshift quasars indicate that the faint radio sources sometimes associated with optically selected quasars do not have larger angular sizes than the more luminous radio-selected sources.

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1. Smith, M. G. *Astrophys. J. Lett.* **206**, L125–L127 (1976).
2. Osmer, P. S. & Smith, M. G. *Astrophys. J.* **210**, 267–276 (1976).
3. Gearhart, M. R. & Pacht, E. *Nature* **266**, 819–822 (1977).

Martian extratropical cyclones

VIKING Orbiter 1 has recently photographed two cloud formations in the northern hemisphere of Mars which closely resemble satellite pictures of terrestrial extratropical cyclones. The pictures, (Figs 1 and 2) were acquired during the summer season and are the first evidence that baroclinic waves, prevalent during other seasons, also occur in summer. Physical properties of these systems deduced from the vidicon images and from the IR thermal mapping experiment (IRTM) are discussed here.

The north polar region of Mars has long been recognised as one of the most meteorologically active areas on the planet, particularly during winter when the polar cap is obscured by a diffuse hood of condensate clouds. Mariner 9 acquired several images of the region in late winter, $L_s = 342^\circ$ to $L_s = 354^\circ$, which have been analysed in detail by Briggs and Leovy¹. The development of the clouds observed in the region surrounding the seasonal polar cap indicated a circulation pattern consistent with baroclinic waves, which had been predicted previously by Mintz².

Viking Lander 2, situated at 48° latitude, just at the edge of the seasonal cap at its largest extent, has monitored local meteorology for more than one martian year; results of a large part of this mission have recently been reported by Ryan *et al.*³. During the autumn season periodic changes are observed in the pressures and wind vectors obtained by VL-2 (but not by VL-1, located in the equatorial region) which strongly suggest the passage of baroclinic systems to the north of the lander site. These variations were obscured by diurnal variations during the first summer, and the major winter dust storm strongly interfered with the activity.

Viking orbiters were not designed primarily as weather satellites; monitoring for meteorology could only be conducted when the spacecraft were near their respective apoapses because of the relatively large focal lengths of the telescopes. Such monitoring was severely restricted by orbital constraints and by competition for a limited number of frames with several other activities. During the latter part of the Extended Mission and the



Fig. 1 The martian storm system observed by the Viking Orbiter 1 at $(81, 160)$, $L_s = 105^\circ$.

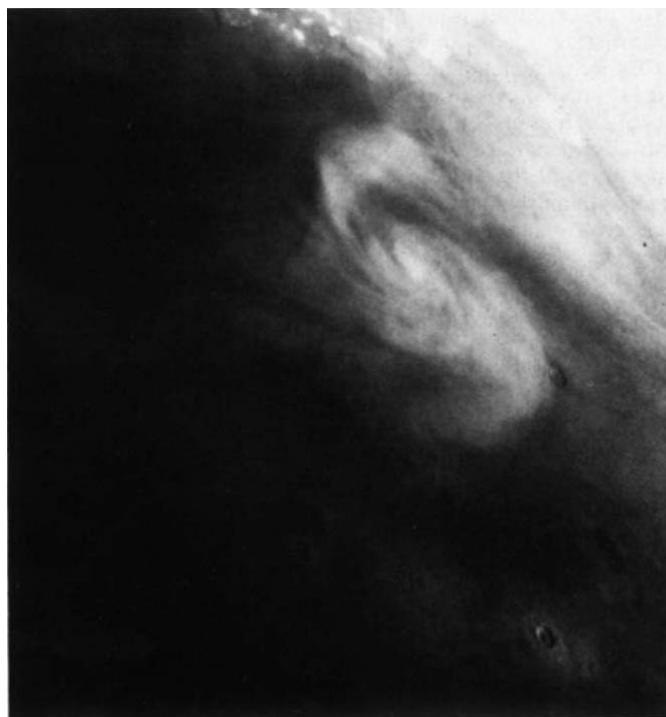


Fig. 2 The martian storm system observed by the Viking Orbiter 1 at $(66, 227)$, $L_s = 126^\circ$.

Continued Mission VO-1 has been able to perform this monitoring in the north polar region; these two pictures represent a small part of that data set, which characterises the spring and summer seasons in the martian northern hemisphere.

The basic parameters which characterise the storms are given in Table 1. These include the areocentric longitude of the Sun (L_s), the coordinates, the local time of day, and an estimate of the radius of curvature. IRTM data relevant to the storms are presented in Table 2 in the form of brightness temperatures in the five IRTM channels. The 7, 9, 11, and $20\text{ }\mu\text{m}$ channels generally characterise the ground temperatures with small differences produced by dust and condensates in the atmosphere⁴; the $15\text{ }\mu\text{m}$ temperatures are characteristic of the atmosphere at a pressure of 0.6 mbar, equivalent to an altitude of about 25 km. Only limited IRTM data are available for the second storm, owing to the geometry and spatial resolution of the scans.

The IRTM measurements show that a large temperature gradient existed just north of storm 1; the magnitude of this gradient was $\sim 0.2\text{ K km}^{-1}$, and the change was confined to about 60 km. The area occupied by the spiral cloud is generally characterised by a ground temperature of $\sim 230\text{ K}$, while temperatures of 215 K are found adjacent to this area to the north. To the south of the storm, between 75° and 80° latitude, there is a gradual rise in the temperature to 237 K. These are precisely the effects which are expected for such a system since the waves which give rise to these disturbances occur in the polar front which separates the frigid arctic air from the warmer mid-latitude atmosphere. The temperature gradient is confined to the lower atmosphere where the clouds are located since there is no structure in the $15\text{ }\mu\text{m}$ data; this is generally expected as only the major dust storms seem to produce pronounced effects at the 0.6 mbar level⁶.

Table 1 Location and scales of the extratropical storms

Storm	L_s	Lat.	Long.	LT	R (km)
1	105°	81°	160°	10.00	100
2	126°	66°	227°	06.33	300

The observed brightness temperatures clearly show that the atmosphere is too warm for the observed cloud to be composed of CO₂ ice particles. The temperature difference between the 9 and 11 μm channels is sensitive to the cloud composition^{4,5}; the negative values of $T_9 - T_{11}$ seen over much of the region suggest a generally dusty background atmosphere, consistent with the appearance of the vidicon images. However, for observations which apparently include the cloud, values of $T_9 - T_{11}$ which are near zero or slightly positive are seen; this strongly suggests a water ice composition for the cloud. Since the interpretation depends on the vertical thermal structure of the atmosphere and as the IRTM resolution is large compared with the detail of the picture, this information is probably not definitive. However, there are several other facts which are consistent with condensate (water) rather than dust.

(1) The albedos associated with the clouds are about 0.3, higher than the surrounding ground and higher than expected for dust.

(2) Comparison of the image in Fig. 1 with a picture taken through the red filter at the same time shows a much larger contrast between cloud and ground in violet than in red; this generally suggests condensate as opposed to dust, as the clouds in the former case are expected to be much more spectrally flat than martian ground or dust.

(3) The water vapour content of the atmosphere is maximum over the north polar region during this season⁷. However, as mentioned, there seems to be dust generally mixed in the atmosphere; the dust may enhance the cloud formation by nucleating the condensation of ice particles to form clouds as air parcels are cooled. This mechanism, the stirring up of surface dust and subsequent nucleation, may be quite important in the seasonal recycling of atmospheric water vapour and in the ultimate formation of the hood.

A dusty atmosphere is also suggested by $T_9 - T_7$, although the 7 μm data points are not conveniently situated for computing this difference in the vicinity of the storm. There is a suppression of the magnitude of the brightness temperature for those IRTM points identified as cloud; this is anticipated, as the increased opacity implies a higher origin for radiation. Unfortunately, two colour coverage of the second storm is not available. However, the limited IRTM data suggest that this cloud is also composed of water ice. The geometry of the scans, and the proximity of the terminator, preclude observation north of this storm, so that there are no direct measurements of expected local temperature gradients.

Table 2 IRTM of the storms (K)

Storm 1	T_7	T_9	T_{11}	T_{15}	T_{20}
Storm area	236	233	233	177	232
Area to north	221	217	218	177	216
Storm 2					
Storm area	216	215	214	167	213

The geometry of the scans prevents observations of the region north of storm 2.

The wind velocity, the pressure gradient, and the radius of curvature determine the force balance for parcels in the storms. The resulting gradient wind, for which the pressure gradient force, the Coriolis force, and the centrifugal force are in balance, is given by

$$v = -fR/2 + ((fR/2)^2 + R|\nabla p|/\rho)^{1/2}$$

where R is the radius of curvature, ∇p the pressure gradient, ρ is the density, and f is the Coriolis parameter, roughly the same for Mars as for Earth. The signs are chosen to represent the normal counter-clockwise circulation around a low pressure cell as seems to be indicated by visual interpretation. VL-2 data were used to estimate the appropriate values of mean pressure and of pressure fluctuations to be used

in the formula; a value of $\nabla p = 0.25$ for the amplitude of the pressure difference seems to be a reasonable upper limit which may be used to estimate an upper limit for v .

The resulting wind velocities and accelerations are given in Table 3. The resulting velocities, 31.5 m s^{-1} and 23.1 m s^{-1} for storms 1 and 2 respectively, lead to surface wind speeds of 12.6 m s^{-1} and 9.2 m s^{-1} if a scale factor of 0.4 is used to interpolate between surface winds and winds at the top of the boundary layer¹⁸. These are certainly reasonable when compared with the typical wind speeds observed by VL-2, and the consistency of the force balance supports the interpretation of these systems taken here. The more northerly storm 1, because of its small size, is approximately cyclostrophic while the larger storm 2 is approximately geostrophic¹¹. The predicted near surface wind speed of $\sim 12.6 \text{ m s}^{-1}$ for storm 1 is close to the saltation velocity (Greeley, personal communication), which is consistent with our interpretation that dust is mixed with the ice particles in the atmosphere.

Table 3 Meteorological properties of the storms

Storm	v (m s^{-1})	p/ρ (nT kg^{-1})	fv (nT kg^{-1})	v^2/R (nT kg^{-1})	Vorticity
1	31.5	0.0144	0.0044	0.0100	6.3×10^{-4}
2	23.1	0.0048	0.0030	0.0018	1.5×10^{-4}

In the case of storm 2, cloud shadows are seen which may be used to estimate the altitude of the clouds. This estimate leads to a height of $\sim 6\text{--}7 \text{ km}$ for this system which is similar to the altitudes deduced for many other martian clouds although some clouds have been seen which are 50 km above the surface^{9,10}. This altitude is quite compatible with the temperature suppression effect mentioned earlier: if a linear lapse rate is assumed, the brightness temperatures observed for cloud 1 are appropriate for an altitude of about 4 km, implying that the intervening material is at least that altitude.

In conclusion, two storm systems observed by Viking seem to be similar to terrestrial mid-latitude cyclonic storms in appearance. Water ice is the favoured composition for the clouds. The scales are somewhat different than for terrestrial storms, but this may be associated with the northerly location. Further work is underway to combine these data with other information acquired by the orbiters to prepare an integrated picture of meteorological phenomena in the Mars north polar region.

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- Briggs, G. & Leovy, C. B. *Bull. Am. met. Soc.* **55**, 278–296 (1973).
- Mintz, Y. in *The Atmospheres of Mars and Venus* Publ. 944 (National Academy of Sciences–National Research Council, Washington, D.C., 1961).
- Ryan, J. A. *et al. Geophys. Res. Lett.* **5**, 715–718 (1978).
- Hunt, G. E. *J. geophys. Res.* (in the press).
- Kieffer, H. H. *et al. J. geophys. Res.* **82**, 4249–4291 (1977).
- Martin, T. Z., Peterfreund, A., Miner, E. D., Kieffer, H. H. & Hunt, G. E. *J. geophys. Res.* (in the press).
- Farmer, C. B., Davies, P. W., Holland, A. L., La Porte, D. D. & Doms, P. E. *J. geophys. Res.* **82**, 4225–4248 (1977).
- Leovy, C. B. & Zurek, R. *J. geophys. Res.* (in the press).
- Anderson, E. & Leovy, C. B. *J. atmos. Sci.* **35**, 723–734 (1978).
- Briggs, G., Klaassen, K., Thorpe, T., Wellman, J. & Baum, W. *J. geophys. Res.* **82**, 4121–4150 (1977).
- Holton, J. T. *Introduction to Dynamical Meteorology* (Academic, New York, 1972).

Differential aeolian redistribution rates on Mars

THE surface of Mars gives abundant evidence for aeolian activity, including wind-laid dust and ice deposits at the poles that may be several kilometres thick, a vast dune field surrounding the north polar deposits, a dust mantle of varying thickness covering the high to mid-latitudes, and both depositional and deflationary aeolian landforms in the equatorial latitudes. Two major questions concerned with the martian aeolian activity are: first, what are the rates of erosion and redistribution of surface materials such as rock and soil deposits; and second, are most of the large-scale aeolian features seen on Mars products of the present aeolian environment, extrapolated back through geological time, or were most of the features produced in a different climatic regime? Presumably, a different climatic regime, either associated with an early, denser atmosphere, or with periodic variations in atmospheric conditions induced by obliquity changes, would strongly modulate the vigour of aeolian activity¹. The Viking Orbiters and Landers, which began acquiring data during the summer of 1976, have now monitored the aeolian environment for slightly more than one full Mars year. Data acquired during the mission have enabled us to investigate these questions for aeolian effects averaged over billions of years, tens of thousands of years, and during a single year.

The integral effect of aeolian activity can be sampled over geological time scales by examining the degree of modification of features large enough to be seen by the Viking Orbiters. Figure 1a shows an orbital view over the Viking Lander 1 (VL1) site. VL1 is sitting on terrain which from orbit seems similar to a lunar *mare* surface. Crater counts by Dial³ show that the crater population of this region is in production, at least for crater sizes extending down to 100 m. Craters smaller than that size are less abundant than would be predicted for a population in production. The deficiency of small craters is probably due to information loss, as the image ground resolution is 25 m per line-pair and a feature typically needs to be several times that size to be detectable. Thus, the crater count data constrain the integral effects of aeolian activity over the lifetime of the crater population to values small enough to have only obliterated craters smaller than 100 m in diameter.

Figure 1b is a VL1 frame showing a crater on the horizon. The crater can also be seen on the orbital mosaic. It is about 2 km from the Lander and it is about 420 m in diameter. The rim height to crater diameter ratio for this feature is about 0.12, which compares favourably with the same ratio for the lunar South Ray crater located at the Apollo 16 landing site. The ratio for South Ray crater, which is 640 m in diameter and 2 Myr old, is 0.09 (ref. 4). South Ray is young enough to be virtually untouched by lunar erosion. The similarity in ratios for the two features strongly implies that the crater in Fig. 1b is also preserved virtually intact. In addition, examination of the orbital mosaic shows that all other craters of similar size have raised rims and bowl-shaped floors. No spectrum of morphologies can be discerned, implying that erosion and deposition have been minimal over the lifetimes of these craters. The rim height of the crater in Fig. 1b is 25 m, so the extent of removal of material must have been some small fraction of that number. Such a conclusion is consistent with the crater count data, because the extent of rock breakdown removal has most probably been a few metres over the lifetime of the crater population.

The extent of preservation of the crater population provides excellent data for estimating that lifetime. From our own crater counts we find that there are $\sim 1.5 \pm 0.2 \times 10^3$ craters per 10^6 km² larger than 1 km in diameter within a radius of 100 km of the landing site. Neukum and Wise⁵ developed a cratering flux model for Mars by assuming that the flux of impacting objects is the same for Mars and the Moon. They also find after

scaling for impact velocity and gravity effects, that the resultant cratering rate would be 0.22 that of the Moon's rate. Such a model results in an age of about 3,600 Myr for the region surrounding VL1. Given the uncertainties of the martian cratering history the actual age could be a factor of 2 or 3 younger than this estimate⁶. However, for any reasonable cratering flux model, the computed erosion rate of metres per 10^9 yr is surprisingly small, some 10^{-3} $\mu\text{m yr}^{-1}$, or ~ 1 m per 10^9 yr.

From both orbital and lander views, the VL2 landing site seems quite distinct from the VL1 site⁷. Extensive evidence for differential deflation can be seen from orbit (Fig. 2a,b). The more resistant areas consist of ejecta deposits from craters, while the deflated regions were probably once covered with fine-grained aeolian deposits that were part of the extensive mantle of deposits surrounding the north pole. Relatively rapid

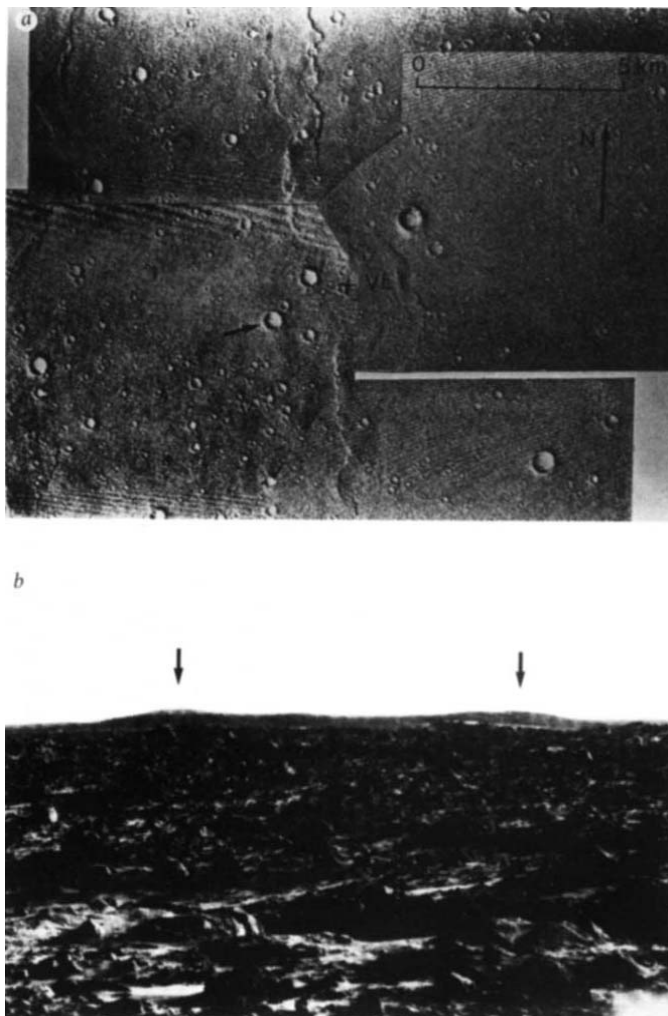


Fig. 1 a, Viking Orbiter 2 mosaic of high resolution frames showing the Viking 1 landing site in Chryse Planitia. ⁺ The Lander location as derived by Morris *et al.*² and updated by Jones (personal communication). The arrow points to the crater that can be seen in the Lander view shown in b. Crater counts by Dial³ show that the crater population is in production at least down to the counting resolution limit of ~ 100 m. This result, together with the fresh-looking appearance of the craters and wrinkle ridges suggests that not more than metres of material have been stripped away over the lifetime of this surface. For reference, VL1 is located at 22.5 N, 48.0 W (ref. 2). VO II frames 452B8–12. b, Viking Lander 1 view of the crater delineated by an arrow in a. The crater is about 2 km from the Lander and 420 m in diameter. The rim height to diameter ratio for this crater indicates that little to no erosion has occurred since its formation. For scale, the bottom of the frame covers 3.3 m. VL1 frame A235.

wind erosion of regions not covered by craters and ejecta have left the latter features standing above most of the terrain as plateaus or pedestals. Ejecta deposits may be more resistant to erosion because they are protected by a population of blocks. Blocky ejecta surfaces may form as a result of ejecta emplacement in a debris flow mode⁸. During debris flows, larger blocks are preferentially forced to the surface because of a vertical dispersive pressure within the flow that scales as the square of the block diameter⁹. Thus, a debris flow mode of ejecta emplacement may create a ready-made armoured surface that lasts for a considerable length of time, given the very low rate of rock removal computed from VL1 data.

The extent of deflation between ejecta and surrounding terrain in the vicinity of VL2 is several hundred metres, assuming a conservative estimate of 10° for the slope of the talus between

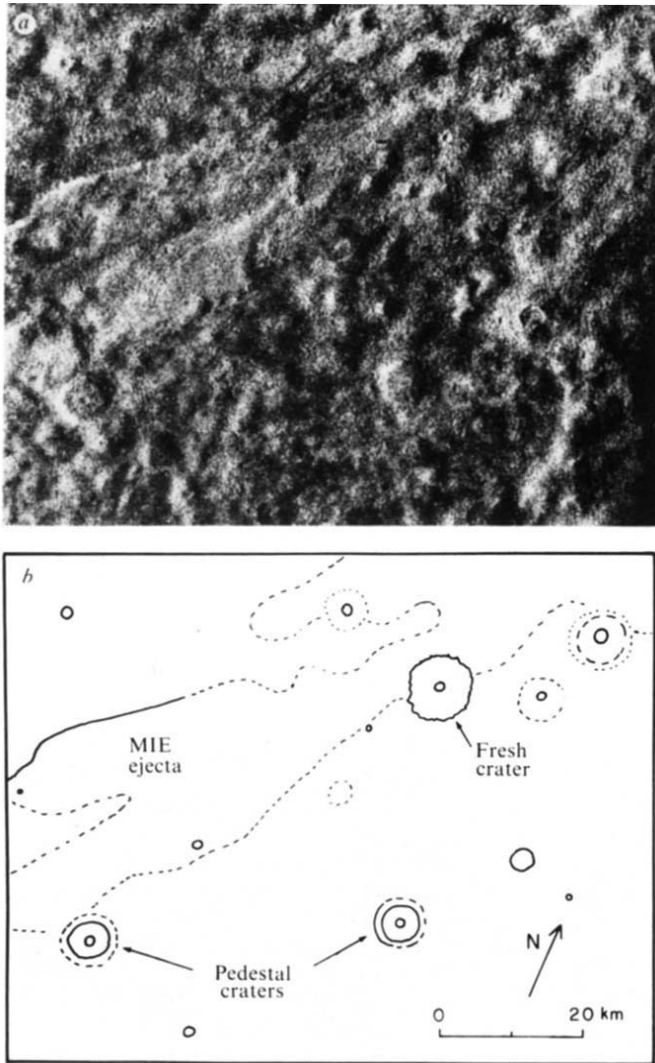


Fig. 2 *a, b*, Viking Orbiter 2 photograph and sketch map of part of Utopia Planitia. Viking Lander 2 touched down in an area that is just off the upper left edge of the frame. The frame is 90 km across. VL2 is located at 48.0° N, 225.6° W, within the northern mantle of aeolian debris surrounding the eroded polar cap deposits. Only one crater with a sharply raised rim, deep, bowl-shaped floor and extensive ejecta unit can be seen. It is shown on the sketch map as a fresh crater. Most other craters have a pedestal-like appearance. The tongue-shaped plateau extending across the upper part of the frame is a lobe of ejecta from the 100 km diameter crater, Mie, located ~ 200 km to the north-east. The topography of this region is controlled largely by differential deflation; high areas consist of craters and ejecta deposits that have resisted erosion. The deflated material probably consisted of fine-grained aeolian sediments associated with the debris mantle. The fresh crater must post-date any period of extensive deflation. VO II frame 9B16.

the edge of the pedestal craters shown in Fig. 2*a, b* and the surrounding deflation horizon. The antiquity of the terrain surrounding VL2 can be estimated, to a first approximation, from the superimposed crater abundances. An age estimate based on the crater population ($1.4 \pm 0.2 \times 10^3$ craters per 10^6 km² larger than 1 km) shown in Fig. 2*a* and surrounding areas would be about 3,400 Myr with the flux model used for the VL1 site. The average deflation rate would thus be very roughly 100 m per 10^9 yr, which is less than $1 \mu\text{m yr}^{-1}$. The estimate is several orders of magnitude greater than the rate of rock erosion derived for the VL1 site and, by implication, for the armoured ejecta deposits at the VL2 site.

Constraints can also be imposed on the integral effect of aeolian activity averaged over time scales of tens of thousands of years. Windblown drifts seen at both landing sites have morphologies indicative of accumulation during a prevailing north to south wind regime—the situation prevalent during the annual global dust storms occurring near the time of perihelion^{10,11}. Surface winds during the global perihelion storms seem to carry near surface dust in a net north to south direction in areas to the north of the subsolar latitude at perihelion (the SLP; presently located at $\sim 15^\circ$ S lat.) and in a net south to north direction in areas more southerly than the SLP. A complex but generally west to east flow seems to dominate the SLP proper¹¹. Most of actual entrainment of dust that feeds the global dust clouds seems to be concentrated in a wide latitude belt centred about the SLP, giving rise to the speculation that the major dark areas of Mars, which roughly coincide with the SLP, have been stripped of a thin, bright dust cover¹². That dust cover would now reside in other regions in the form of deposits such as the drifts at VL1 and VL2. Due to precessional effects, the SLP migrates $\pm 25^\circ$ in latitude about the Equator over a 50,000 yr time span¹³. Because of the enhanced stripping associated with regions close to the SLP, it seems probable that the drifts at VL1, with their strictly north to south trend, must have accumulated since the SLP was last over the landing site latitude (24° N lat.), which would have been about 15,000 yr ago. Presumably drifts with west to east trends, or perhaps a stripped surface, would have dominated the surface at that time. If this line of reasoning is accepted, then a net accumulation rate of about 10 cm (mean drift thickness) per 15,000 yr, or several $\mu\text{m yr}^{-1}$, can be computed.

Figure 3 shows a pair of Viking Lander 2 (VL2) pictures acquired 476 martian days apart. The striking difference is related to the deposition of a thin layer of bright dust during the second major dust storm that occurred during the Viking mission. The dust is detectable only by its increased brightness; even then, it is only detectable in pictures acquired at high Sun angles (low phase angles). This constraint implies that the dust layer is probably only micrometres thick¹⁴. So far, no significant erosion of the new dust deposit has taken place, although such an accumulation, without subsequent erosion, cannot occur over more than a few tens of thousands of years without burying the boulders exposed at the landing site. Conditions at VL1 have been similarly quiet, with the accumulation of a bright dust layer, again probably only micrometres in thickness, being the dominant activity¹⁴. Thus, both landing sites have experienced an aeolian environment that, averaged over a yearly time scale, has resulted in the redistribution of several micrometres of dust. Such a conclusion is consistent with Lander camera observations of atmospheric opacity during the same time frame, which suggest dust transport rates of about 3×10^{-4} g cm⁻² yr⁻¹ (ref. 15). Deposited evenly over the surface, such a rate equates to a dust layer of about $1 \mu\text{m}$ in thickness.

Our results for the Viking Landing sites indicate that little rock breakdown and removal by wind has occurred over approximately the past 3,000 Myr. On the other hand, relatively rapid redistribution of friable or loose, fine-grained materials has occurred. Many of the landforms seen in higher resolution Viking Orbiter frames covering other parts of Mars are consistent with such interpretations. Many volcanic flow fronts, well preserved crater populations, and other features which are

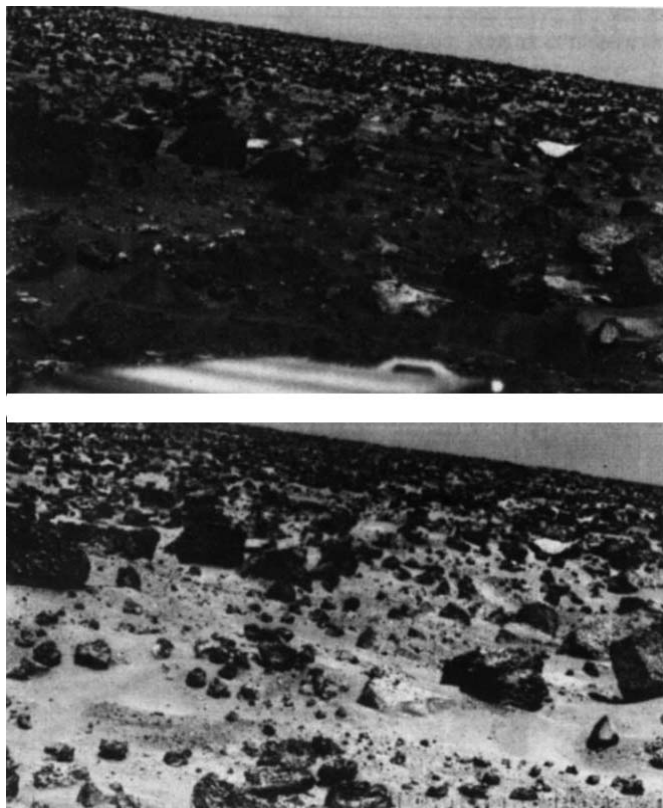


Fig. 3 The top VL2 frame was acquired 32 d after landing, during the Northern Hemisphere's summer season. The bottom frame was acquired 476 d later, with identical lighting. The brightness difference is related to an accumulation of several micrometres of bright dust during the second global dust storm that occurred during the martian year of Viking observations. The top frame is B013 and the bottom is G052.

indicative of little to no significant modification can be discerned. In other regions, the surface has been stripped, yardangs have formed, and in general the topography seems to have been largely configured by aeolian activity. Such regions must be composed of friable deposits, such as volcanic ash or older aeolian materials. Finally, the similarity of redistribution rates for aeolian deposits averaged over three vastly different time-scales implies that roughly the present aeolian environment has been operative for a significant fraction of geological time.

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1. Ward, W. R., Murray, B. C. & Malin, M. C. *J. geophys. Res.* **79**, 3387–3395 (1974).
2. Morris, E. C., Jones, K. L. & Berger, J. P. *Icarus* **34**, 548–555 (1978).
3. Dial, A. L. *Rep. planet. Geol. Program*, 1977–1978, 179–181 (NASA TM-79729, 1978).
4. Arvidson, R. E., Crozaz, G., Drozd, R. J., Hohenberg, C. M. & Morgan, C. J. *The Moon* **13**, 259–276 (1975).
5. Neukum, G. & Wise, D. U. *Science* **194**, 1381–1387 (1976).
6. Shoemaker, E. M. & Helin, E. F. *Rep. planet. Geol. Program*, 1976–1977, 74–77 (NASA TMX-3511, 1977).
7. Mutch, T. A., Arvidson, R. E., Binder, A. B., Guinness, E. A. & Morris, E. C. *J. geophys. Res.* **82**, 4452–4467 (1977).
8. Carr, M. H. *et al. J. geophys. Res.* **82**, 4055–4065 (1977).
9. Bagnold, R. A. *Proc. R. Soc. A* **225**, 49–63 (1954).
10. Veverka, J., Cook, K. & Goguen, J. *Icarus* **33**, 466–482 (1978).
11. Arvidson, R. E. *Icarus* **21**, 12–27 (1974).
12. Soderblom, L. A., Condit, C. D., West, R. A., Herman, B. M. & Kreidler, T. J. *Icarus* **22**, 239–263 (1974).
13. Ward, W. R. *Science* **181**, 260–262 (1973).
14. Jones, K. L. *et al. Science* (in the press).
15. Pollack, J. B. *et al. J. geophys. Res.* (in the press).

Gravitational magnetism

BLACKETT¹ has pointed out that the ratios of magnetic moment to angular momentum for Earth, the Sun, and the star, 78 Virginis, are nearly equal and of a magnitude which suggests a gravitational origin for the magnetic fields of these bodies. After his laboratory experiment testing this hypothesis yielded negative results², gravitational magnetism became largely ignored. Recent magnetic field measurements of the moon³, Mercury⁴, and Jupiter⁵, however, yield ratios quite close to that considered by Blackett. Moreover, Russell⁶, who emphasises only the phenomenology of these ratios and eschews any physical model, adds Venus and Saturn to the list, although these data are tentative and will become firm only with the completion of probes to Venus and Saturn. His re-evaluation of Mars, however, puts this planet into an exceptional position as it seems to have no measurable magnetic field. I agree with his suggestion that to account for this we must ultimately consider the interiors of the planets. Hopefully more precise magnetic measurements will be made on Mars. Meanwhile a new data point strengthens the gravi-magnetic hypothesis: the magnetic field of a pulsar—Hercules X-1—has been directly measured^{7,8} and yields the Blackett ratio. Because the Hercules data is both fairly exact and represents an extremely high magnetic field (5×10^8 T) and angular velocity (5 rad s^{-1}), it is appropriate to reconsider Blackett's gravi-magnetic hypothesis, and I show here that the gravi-magnetic hypothesis has yet to be tested definitively in the laboratory.

The gravi-magnetic hypothesis proposes that a rotating mass, measured in gravitational units, has the same magnetic effect as that of a rotating charge, measured in electrical units. The respective force constants determine this relationship

$$G^{1/2}m = k^{1/2}q \quad (1)$$

where G is the gravitational constant, m is mass, k is the Coulomb constant, and q is electric charge. Thus the ratio of magnetic moment to angular momentum for a sphere of mass, m , density factor, f , radius, r , angular velocity, ω , and magnetic field, B , is (in SI units)

$$P/U = 5/4 \pi B/\mu_0 \quad r/f\omega m = G^{1/2}/2k^{1/2} \quad (2)$$

Table 1 and Fig. 1 show the degree to which this expectation is met: the data points in Fig. 1 fit the $G^{1/2}/2k^{1/2}$ line remarkably well.

Note that the mere fact of a linear relationship between the magnetic moments and angular momenta is not what is surprising in these data. *A priori*, we should expect a correlation between P and U . In equation (2) we expect r to be correlated with m , and B to be correlated with ω ; and thus, we expect P to be correlated with U . The surprise is that this correlation ratio, P/U , should turn out to be close to $G^{1/2}/2k^{1/2}$. Without the gravi-magnetic hypothesis, there is no reason to expect the regression line for the data of Fig. 1 to be close to $P = G^{1/2}/2k^{1/2} U$. If we have only one datum point, and it has a P/U close to $G^{1/2}/2k^{1/2}$, we should be surprised enough to take notice of the gravi-magnetic hypothesis. However, that one datum point may not turn out to be typical. The value of Fig. 1 is in showing that the data points typically fall close to the gravi-magnetic hypothesis line. The only atypical point, thus far, is Mars. The gravi-magnetic hypothesis (stated in $\log_{10}$ form) predicts a P/U of -10.37 . The mean P/U for the data points plotted in Fig. 1 is -11.13 with a standard deviation of 0.42 .

As we have no *a priori* way of knowing the likelihood of the near coincidence of P/U with $G^{1/2}/2k^{1/2}$, it may be useful to calculate how easily the data points can be pulled away from the gravi-magnetic hypothesis line. To do this, we must take into account the interdependence of the variables: r , m , B , and ω . Thus, if one changes r , the other variables would be expected to change in accordance with this dependence. There are two

Table 1 Maximum and minimum data for ratio of magnetic moment to angular momentum for massive bodies

Body	f	m Mass	r Radius	ω Angular velocity	B Magnetic field	U Angular momentum $\frac{2}{5} f \omega m r^2$	P Magnetic moment $\frac{1}{2} \frac{4\pi}{\mu_0} B r^3$	P/U $\times 10^{-11}$ (C kg ⁻¹)	$\beta^\dagger$ $\frac{P}{U} \div \frac{G^\dagger}{2k^\dagger}$
(refs)		(kg)	(m)	(rad s ⁻¹)	(T)	(Js)	(A m ²)		
Earth (1, 15–17)	0.88	5.98×10^{24}	6.378×10^6	7.272×10^{-5}	7.0×10^{-5} 2.5×10^{-5}	6.22×10^{33}	9.1×10^{22} 3.2×10^{22}	1.5 0.51	0.35 0.12
Sun (15, 16, 18, 19)	0.16	1.99×10^{30}	6.960×10^8	2.9×10^{-6} 2.7	27×10^{-4} 0.8×10^{-4}	1.79×10^{41} 1.67×10^{41}	4.6×10^{30} 0.14×10^{30}	2.7 0.078	0.63 0.02
78 Vir (1–3)	0.16	4.8×10^{30} 4.4×10^{30}	1.8×10^9 1.0×10^9	5×10^{-4} 0.5×10^{-4}	0.2 0.1	5.0×10^{44} 0.14×10^{44}	5.8×10^{33} 0.5×10^{33}	41 0.1	9.8 0.02
Moon (3, 15, 16)	0.88	7.35×10^{22}	1.738×10^6	2.66×10^{-6}	10.3×10^{-8} 3.8×10^{-8}	2.08×10^{29}	2.7×10^{18} 1.0×10^{18}	1.3 0.48	0.30 0.11
Mercury (4, 6, 15)	0.88	3.3×10^{23}	2.434×10^6	1.24×10^{-6}	4×10^{-7} 2×10^{-7}	8.5×10^{29}	2.8×10^{19} 1.4×10^{19}	3.3 1.6	0.77 0.37
Venus (6, 15)	0.88	4.87×10^{24}	6.052×10^6	2.99×10^{-7}	5.9×10^{-8} 2.7×10^{-8}	1.88×10^{31}	6.5×10^{19} 3×10^{19}	0.35 0.16	0.08 0.04
Jupiter (5, 6, 15)	0.66	1.90×10^{27}	7.143×10^7	1.773×10^{-4}	14×10^{-4} 3×10^{-4}	4.54×10^{38}	26×10^{26} 5.5×10^{26}	0.57 0.12	0.13 0.03
Saturn (6, 15, 20)	0.66	5.69×10^{26}	5.98×10^7	1.706×10^{-4}	3×10^{-4} 1×10^{-4}	9.16×10^{37}	3.2×10^{26} 1.2×10^{26}	0.35 0.13	0.08 0.03
Her X-1* (7, 8, 21, 22)	0.88	3.5×10^{30}	1×10^4 0.7×10^4	5.076	7×10^8 5×10^8	3.1×10^{38} 1.8×10^{38}	35×10^{25} 8.6×10^{26}	1.9 0.28	0.44 0.07

* For neutron stars, the larger mass is associated with the smaller radius²¹.

† $G^{1/2}/2k^{1/2} = 4.3 \times 10^{-11} \text{ C kg}^{-1}$.

measures of this dependence. First, each datum point has its own dependence relations. Second, there is a set of mean dependence relations for the data as a whole. Thus for all the data points (except 78 Vir and Her X-1)—in log₁₀ form, and in SI units—the mean of the ratio m/r is 3.57 (s.d. = 0.11); the mean B/r is -0.726 (s.d. = 0.35); the mean ω/r is -0.701 (s.d. = 0.21).

If one changes r for each datum point (except 78 Vir and Her X-1) by an order of magnitude, and changes m , B , and ω according to the individually calculated dependence relations, P/U for each datum point will change by more than two orders of magnitude. The mean P/U for these changed data points will be -13.95 (s.d. = 0.47), compared with the mean P/U of -11.18 for the unchanged data points.

A greater change than this will occur if one uses the mean dependence relations mentioned above. In this case the mean P/U for the changed data points will be -14.20 (s.d. = 2.21).

Since r in Table 1 ranges over five orders of magnitude, a change in r of one order of magnitude is relatively small, and thus we can say that the data points are sensitive to relatively small changes in the relevant variables.

A striking characteristic of the data in Fig. 1, is that the deviation from the gravi-magnetic hypothesis line is fairly systematic. It should be obvious, of course, that deviations from the $G^{1/2}/2k^{1/2}$ may well be due to electrical-magnetic effects. For example, equation (2) predicts a surface field about three times greater than that measured at the surface of the Earth. However, because of the offset between the magnetic and gravitational poles of Earth, electrical-magnetism must also be at work. Whatever accounts for this electrical component of the magnetic field of Earth must also account for the damping of the gravitational component.

The *modus operandi* of the electrical component may also help account for the deep mine measurements of Runcorn^{9,10}, which show the magnetic field increasing towards the centre of the Earth. Blackett² regarded these measurements as decisive evidence against his gravi-magnetic hypothesis, because the gravi-magnetic hypothesis, taken by itself, predicts that the magnetic field will decrease in this direction. This evidence, however, is not decisive. To see this, we must solve equation (2)

for the magnetic field B . The expected magnitude is thus seen to be

$$B = 4/5 \mu_0/4\pi G^{1/2}/2k^{1/2} f \omega m/r \quad (3)$$

For a given body, f and ω remain constant, yielding m and r as the only variables. M , for a uniformly dense sphere, is a linear function of volume— $4/3 \pi r^3$. However fast r falls off in the denominator of equation (3), m in the numerator falls off faster. B thus decreases as r decreases.

To counter the decisiveness of Runcorn's measurements, one must first note that the Earth is not a uniformly dense sphere, so that m is a function of density as well as of volume. As density increases towards the Earth's centre, the decrease in B expected from the gravi-magnetic equation (3) is not as precipitous as the above volume argument might lead one to expect. At the Earth's surface, for instance, equation (3) predicts a B of 2.1×10^{-4} T. This is not, however, a great deal more than the 1.4×10^{-4} T that equation (3) predicts for the surface of Earth's core. More importantly, this core magnetism predicted by the gravi-magnetic equation is greater than the magnetic field of 6×10^{-5} T measured at the Earth's surface. Runcorn^{9,10} measured an increase of about 7×10^{-9} T over Earth's surface field of 6×10^{-5} T, still approximating 6×10^{-5} T, and considerably less than the 2.1×10^{-4} T predicted by gravi-magnetism for Earth's core. This is what we expect if we suppose that gravitational magnetism is modified by an electrical-magnetic effect stronger at the Earth's surface than in the interior. Runcorn's measurements are thus not decisive.

A decisive test for gravitational magnetism must be with laboratory-sized samples. Although Blackett's experiment² gave the appearance of being such a test, I now argue that this and other experiments have not definitively tested the predictions of the gravi-magnetic hypothesis, but only various extra assumptions, which were necessary at the time to predict effects large enough to be seen in laboratory-sized samples.

Wilson¹¹ exploited an ambiguity in the most general form of the gravi-magnetic hypothesis, which states that a mass moving either translationally or rotationally generates a magnetic field, just as a charge moving either translationally or rotationally

generates a magnetic field. There is a great difference, however, between translational and rotational movement. The former is relative, in the sense of special relativity; the latter is not. For charge, a translational movement does set up a magnetic field relative to the motion of the observer, a fact which was the starting point for Einstein's theory of relativity. Thus, although the observational basis for gravitational magnetism is only that that massive rotating bodies possess nearly proportional magnetic fields, Wilson wrongly assumed that equation (3), which predicts the magnitude of the gravi-magnetic effect, could be made completely parallel to the electrical case by writing it in velocity form and assuming this velocity to be relative. Thus he had

$$B = 4/5 \mu_0/4\pi G^{1/2}/2k^{1/2} mv/r^2 \quad (4)$$

Because linear velocity is relative, Wilson could swing an iron bar in the laboratory and with his assumptions, consider that it was the Earth with all its mass that was moving past the bar. He thus put the Earth's mass and radius into equation (4) and expected a measurable field of 10^{-7} T. Wilson found that no field of such magnitude exists for laboratory-sized bodies in linear motion, thus ruling out the linear version of the gravi-magnetic hypothesis.

Swann and Longacre¹² tried a different idea. Swann¹³ had shown that by rewriting equation (4) in density form (where ρ is density)

$$B = 8\pi/15 \mu_0/4\pi G^{1/2}/2k^{1/2} \rho \omega r^2 \quad (5)$$

and replacing $\rho \omega r^2$ in equation (5) with $\rho \omega^4 r^4$, magnetic field ratios of the correct magnitude for Earth and the Sun can be predicted as well as measurable magnetic fields for rotating bodies of small mass. The substitution of $\rho \omega^4 r^4$ for $\rho \omega r^2$, however, makes equation (5) dimensionally incorrect, Swann therefore had to add arbitrary parameters to yield correct magnetic moments. This *ad hoc* procedure yielded a field expectation of about 10^{-7} T for a 20 cm copper sphere spinning at 200 Hz. Swann and Longacre spun such a sphere and showed that no field existed, to the limits of their measuring equipment, which was on the order of 10^{-8} T. Swann's version of equation (5) was thus ruled out. The Swann-Longacre experiment,

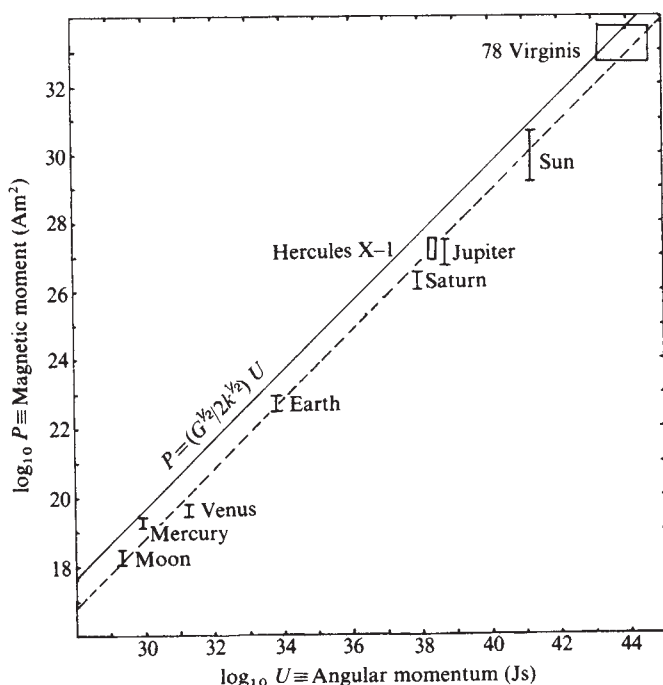


Fig. 1 Magnetic moment/angular momentum diagram. Comparison of gravi-magnetic hypothesis line, $P = (G^{1/2}/2k^{1/2}) U$, with P and U for massive rotating bodies. The dotted line is the regression line for the data. In $\log_{10}$ form, and in SI units: $G^{1/2}/2k^{1/2} = -10.37$; the mean P/U is -11.13 ± 0.42 .

Table 2 Laboratory experiments testing gravitational magnetism

Experiment (refs)	Assumption (equation number)	Expectation (T)*	Sensitivity (T)	Result
Wilson ¹¹ (1923)	Translational effect: field due to mass and radius of Earth (4)	10^{-7}	10^{-8}	0
Swann & Longacre ¹² (1928)	Special rotational effect: $\rho \omega r^2$ becomes $\rho \omega^4 r^4$ (5)	10^{-7}	10^{-8}	0
Blackett ² (1952)	Virtual charge effect: static-body field due to rotation of Earth (4)	10^{-12}	10^{-13}	0
SQUID ¹⁴ (future)	Simple rotation effect: field due to rotating mass of laboratory-size body (3)	10^{-12}	10^{-13}	?

* 1 T = 1 tesla = 10^4 G (of B).

however, did not rule out the simple rotational version of the gravi-magnetic hypothesis, as this version of equation (5) yields a prediction of 10^{-13} T, which was five orders of magnitude below the sensitivity of their measuring apparatus.

After a gap of two decades, Blackett^{1,2} took up the challenge of a laboratory test, encouraged by the new data from 78 Virginis which fit the gravi-magnetic hypothesis. However, Blackett, like Swann and Longacre before him, could not devise an instrument capable of measuring in the range of 10^{-13} T generated by a laboratory-sized spinning body. But he could measure the field of a static body, even if the field was only 10^{-12} T. Blackett therefore investigated a version of the gravi-magnetic hypothesis which predicted a static-body field on the order of 10^{-12} T. According to this version, a rotating body, such as the Earth, by reason of its mass, sets up a virtual electric current, so that a measuring instrument at rest on its surface registers a magnetic field on a small mass which is also at rest; both the small mass and the measuring instrument participate in the Earth's rotation, so that their velocity component, which is due to the Earth's rotation, can be put into equation (4). Thus Blackett calculated a field expectation of 10^{-12} T for a 15 kg mass at rest; and by clever experimental procedures he was able to rule out such a static-body field. He regarded this negative result as a confirmation of Runcorn's results which had been announced just as his own experiment was starting.

Such a catalogue of negative results has perhaps caused physicists to lose sight of the fact that a definitive test of the simple rotational version of the gravi-magnetic hypothesis has never been carried out in the laboratory. A definitive experiment may now be possible and should be attempted. Blackett² himself pointed out that a 1-m bronze sphere spun at 100 Hz (its maximum safe speed) should, on the simple rotational gravi-magnetic hypothesis, yield a field of 10^{-12} T. Such a field can be measured by a SQUID magnetometer¹⁴ limited by an internal field of only 8×10^{-14} T (r.m.s. per root cycle). Table 2 summarises this experimental situation.

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1. Blackett, P. M. S. *Nature* **159**, 658-666 (1947).
2. Blackett, P. M. S. *Phil. Trans. R. Soc. A* **245**, 309-370 (1952).
3. Dyal, P. & Parkin, C. W. *Scient. Am.* **225-2**, 62-73 (1971).
4. Ness, N. F., Behannon, K. W., Lepping, R. P., Whang, Y. C. & Schatten, K. H. *Science* **184**, 151-160 (1974).
5. Wolfe, J. H. *Scient. Am.* **233-3**, 119-126 (1975).
6. Russell, C. T. *Nature* **272**, 147-148 (1978).
7. Coe, M. J., Engel, A. R., Quenby, J. J. & Dyer, C. S. *Nature* **258**, 508-509 (1977).
8. Lenzen, R. & Trumper, J. *Nature* **271**, 216-220 (1978).
9. Runcorn, S. K., Benson, A. C., Moore, A. F. & Griffiths, D. H. *Phil. Mag.* **41**, 783 (1950).
10. Runcorn, S. K., Benson, A. C., Moore, A. F. & Griffiths, D. H. *Phil. Trans. R. Soc. A* **244**, 113 (1952).
11. Wilson, H. A. *Proc. R. Soc. A* **104**, 451 (1923).
12. Swann, W. F. G. & Longacre, A. J. *Franklin Inst.* **206**, 421 (1928).
13. Swann, W. F. G. *Phil. Mag.* **3**, 1088 (1927).

14. Brenner, D., Williamson, S. J. & Kaufman, L. *Science* **190**, 480–481 (1975).
15. Sagan, C. *Scient. Am.* **233**–3, 23–31 (Sept 1975).
16. Ohanian, H. C. *Gravitation and Spacetime* (Norton, New York, 1976).
17. Jensen, H. *Scient. Am.* **208**–6, 161–162 (1961).
18. Starr, V. P. & Gilman, P. A. *Scient. Am.* **248**–1, 100–113 (1968).
19. Livingston, W. C. *Scient. Am.* **215**–5, 54–62 (1966).
20. Kaiser, M. L. & Stone, R. G. *Science* **189**, 285–287 (1975).
21. Smith, F. G. *Pulsars* (Cambridge University Press, 1977).
22. Joss, P. C. & Rappaport, S. A. *Nature* **264**, 219–222 (1976).
23. Harwit, M. *Astrophysical Concepts* (Wiley, New York, 1973).

Low-level air flow over the western Indian Ocean as seen from METEOSAT

DURING the northern summer, the low-level air circulation over the Indian Ocean is characterised by cross-equatorial flow from the Southern to the Northern Hemisphere. The south-east trade winds of the Southern Hemisphere are sucked in by the zone of continental low pressure located over central India and are mainly deflected by the Coriolis force after crossing the Equator; they give rise to the south-west Indian monsoon and associated precipitation during the summer months¹. The cross-equatorial air flow is not uniform at all longitudes: it is weak over the eastern Indian Ocean and particularly strong along the East African coast, where it is concentrated into a low-level jet (maximum intensity 30 ms^{-1} at between 1 and 3 km) flowing to the Somalia coast from the northern tip of Madagascar². We now report the first results from a study of the low-level airflow circulation over the western Indian Ocean and more particularly the Somali low-level jet stream. Successive images taken by the European geostationary satellite METEOSAT were used to determine wind vectors.

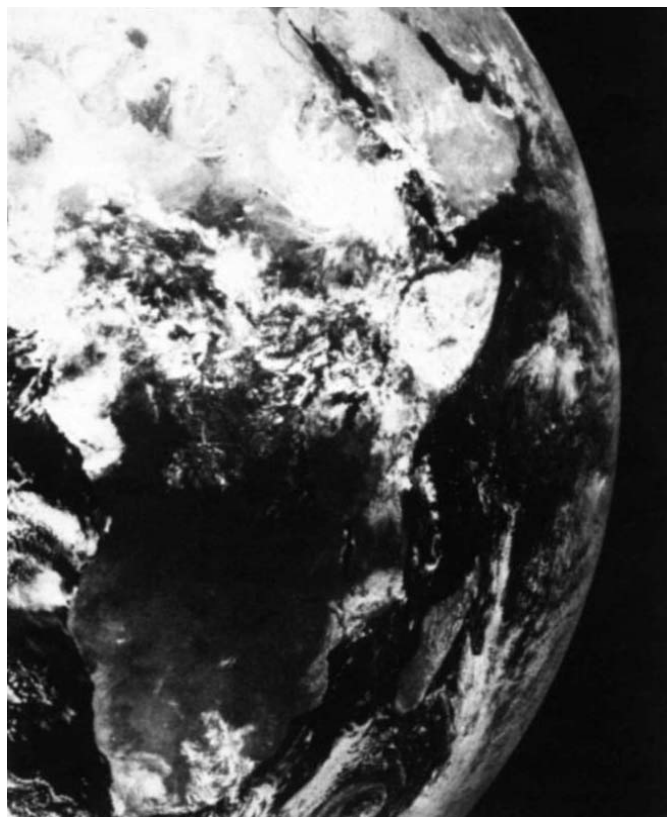


Fig. 1 METEOSAT view of the Indian Ocean on 16 July 1978, at 12.00 GMT.

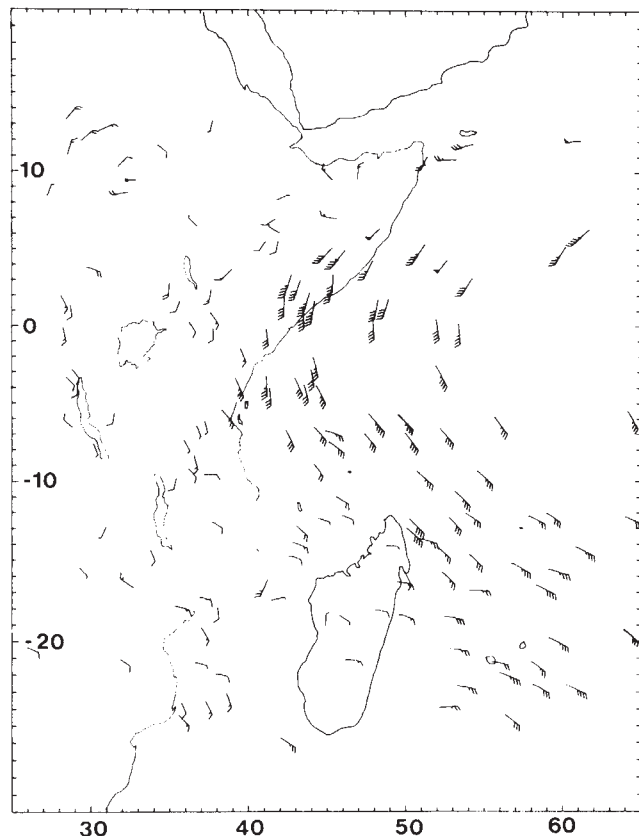


Fig. 2 Wind vectors determined from cloud displacements on 11 July 1978.

The Somali jet can have a regulating effect on monsoon precipitation over the Indian sub-continent, and a correlation has been found between the amount of rainfall over the western coast of India and the intensity of low-level equatorial winds over Kenya². It has also been found that cross-equatorial winds are stronger during a good monsoon year than during a bad one. The complete structure of this jet is largely unknown because it flows mainly over the ocean. For example, fluctuations of its intensity over Kenya, where it can be studied, may reflect a longitudinal shift of the core of the jet rather than a temporal variation of the intensity. The structure of the jet was intensively studied with an instrumented aircraft for a few days during MONEX-77, but no long-term investigation of variations and geographical extension has been made—this would be particularly difficult with conventional observations because of the marine extension of the jet. However, a geostationary meteorological satellite taking images at intervals would allow such a study from the analysis of cloud displacements. METEOSAT can serve this function and has been working since the end of 1977. It gives a view of the western Indian ocean up to about 60°E and is positioned in the neighbourhood of longitude 0° . METEOSAT produces images of the Earth and its cloud cover every half-hour in three spectral bands—one band in the visible spectrum and two in the IR spectrum (thermal IR, $10.5\text{--}12.5 \mu\text{m}$, and water vapour IR, $5.7\text{--}7.1 \mu\text{m}$, bands) with a ground resolution of 2.5 km for the visible channel and 5 km for the IR channels. From successive images cloud elements can be tracked to infer wind vectors.

The displacement of each cloud element can be determined by using the optical correlator built in our laboratory. This apparatus makes it possible to visualise several successive high resolution images on film loops and to move each image in relation to the others. Pictures can be aligned very precisely by using the landmarks; cloud motions are then measured by moving the pictures in such a way that the observed cloud appears motionless during the film loop; the relative displacements of the

pictures and the location of the cloud elements are recorded and the results are processed on a computer that plots them on a map. This method is relatively laborious but is very precise and moreover, the operator himself chooses the cloud element. In this study, three images were used: two successive visible images taken around noon and a thermal IR image taken at the time of the first visible image. The loop is made with the two successive visible images, and the IR one is used to discriminate the level of the clouds.

At low levels, the deviation between cloud vectors and raw wind data is often small—less than 5 knots. Three factors which might contribute to this error are: (1) an error in the tracking of the clouds; (2) a wrong height given to a cloud vector; and (3) a wrong cloud vector due to its displacement by non-advective effects (for example, orographic clouds or clouds related to local thermal effects). The first error is minimal when an optical correlator is used. The second one is the most difficult to exclude: for convective clouds the level of the top can be easily found on the IR picture, but the cloud motion more closely follows the wind near the base of the cloud. When the cloud top is not higher than the 600 m bar level, the wind given is assumed to be representative of the 900 m bar level. This is particularly so over the Indian Ocean where the trade winds are relatively deep. The third kind of error cannot be detected by objective techniques: it is very weak over the ocean but increases over land.

From METEOSAT low-level clouds (cumulus) are clearly visible over the entire marine region except for the northern Somalia coast, where the cold Somali oceanic current inhibits cloud activity (Fig. 1). Intensive convective activity prevails over Madagascar where it is difficult to infer wind vectors due to orographic effects. Over the flat land of Kenya and southern Somalia, wind vectors can be deduced from cloud motions within the low-level jet. Over the African continent and outside the region where the Somali jet is flowing, cloud motions are very weak and it is difficult to infer wind vectors, as the clouds are related to local convective phenomena.

Figures 2 and 3 give examples of raw wind vectors (not interpolated on a grid) as deduced from cloud motions on two days of the 1978 summer monsoon (11 and 16 July). It can be seen that data cover a large part of the western Indian Ocean. The only regions for which there are no data are certain parts of the African continent, the Arabian peninsula and the marine region off the northern Somali coast. The low-level wind field agrees quite well with previous studies based on conventional but scarce data⁴. On 11 July (Fig. 2) the Somali jet is clearly visible. It flows from the east of the northern tip of Madagascar to Africa where it can be seen over the flat lands of eastern Africa before flowing towards India. The results illustrate the importance of the mountainous ranges from Kenya to Ethiopia and of the Madagascar barrier. Strong winds can be seen around the Mascarene Islands showing that the roots of the jet may be found to the east of 60° E. North of the Equator the Coriolis force deflects the air and gives rise to westerlies over the Arabian Sea. An interesting feature of the clockwise gyre of air concerns its geographical extension. Thus on 11 July it was particularly large (larger than deduced from conventional data) and at the Equator strong winds can be found from 40° E to 55° E; the core of the jet is located close to the African coast. Another feature seen in Fig. 2 is a well-defined depression over Sudan corresponding to the early stage of an African easterly wave.

On 16 July (Fig. 3) the wind field was quite different. The Somali jet can also be seen but only to the north of 10° S and it is very weak. An intensification can be noted over the western Arabian sea off the Somali coast. The geographical extension is very weak and the root in the Mozambique channel is stronger than on 11 July. The most intriguing feature is the wind discontinuity found to the east of Madagascar; it seems to be related to the weakening of the low-level jet. This discontinuity corresponds to the normal deflection of the winds by a frontal zone moving westwards in the Southern Hemisphere. As can be seen in Fig. 1, this frontal system is associated with a mid-latitude cyclonic perturbation centered far south of Madagascar.

Thus extra-tropical systems of the Southern Hemisphere may interact with the cross-equatorial flow of the monsoon. This point needs further consideration with a study using wind fields on days around 16 July.

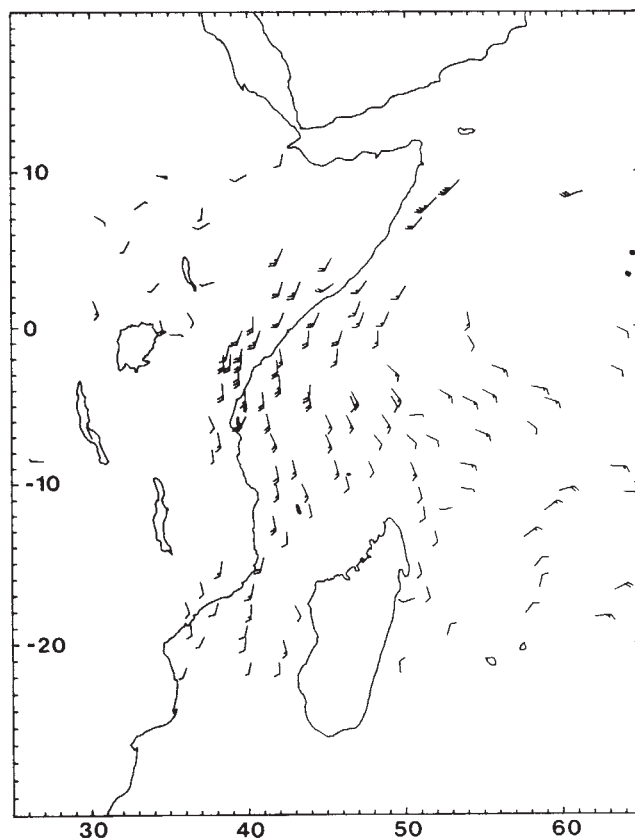


Fig. 3 Wind vectors determined from cloud displacements on 16 July 1978.

Our preliminary study has shown that the large-scale features as well as the temporal fluctuations of the Somali low-level jet can be studied from successive images taken from a geostationary platform. Since a complete view of the western Indian Ocean can be obtained, the causes of the fluctuations may be found. A daily extensive study was performed, covering 15 days in July 1978 as well as the end of May during the onset of the Indian summer monsoon. Our work stresses the value of a geostationary satellite over the western Indian Ocean region. Thus GOES-1, taking images of the Indian Ocean during MONEX-79, will be an important component of the MONEX observational systems.

We are grateful to the European Space Agency for providing us with the METEOSAT images.

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1. Cadet, D. & Ovarlez, H. *Q. Jl R. meteor. Soc.* **102**, 805–816 (1976).
2. Findlater, J. *Q. Jl R. meteor. Soc.* **95**, 362–380 (1969).
3. Krishnamurti, T. N., Molinari, J., Hua Lu Pan *J. atmos. Sci.* **33**, 2350–2362 (1976).
4. Cadet, D. & Olory-Togbé, P. *Q. Jl R. meteor. Soc.* **104**, 971–977 (1978).

Stratospheric aerosol optical thickness measurements at 35°S

In the stratosphere the attenuation in the radiance of a light beam is predominantly due to scattering by air molecules and aerosols, and absorption by ozone. For the height range 10–30 km, aerosols can account for at least 60% of the attenuation at 700 nm (ref. 1) and are responsible for most of the long and short term variations². During 1969–76 the stratospheric optical thickness due to aerosol scattering was measured at Adelaide 35°S, using a lidar³. The observations reported here show that the aerosol optical thickness varied by more than a factor of three during that time.

The transmittance⁴ of the atmosphere, $T(h_1, h_2)$, between heights h_1 and h_2 is a measure of the fractional attenuation of a vertically directed beam and is given by

$$T(h_1, h_2) = T_M(h_1, h_2) T_A(h_1, h_2) T_0(h_1, h_2) \\ = \exp \left[- \int_{h_1}^{h_2} \{ \beta_M(h) + \beta_A(h) + \beta_0(h) \} dh \right] \\ = \exp [- \tau_M(h_1, h_2) - \tau_A(h_1, h_2) - \tau_0(h_1, h_2)] \quad (1)$$

where β_M and β_A are the molecular and aerosol volume scattering coefficients, β_0 the ozone volume absorption coefficient, and τ_M , τ_A and τ_0 are the respective optical thicknesses. For lidar studies it is necessary to introduce the volume scattering function $B(\theta, h)$ which is a measure of the radiation scattered per unit solid angle in the direction θ at the height h in the atmosphere. The total radiation scattered out of a beam is given by

$$\beta(h) = \int_{4\pi} B(\theta, h) d\omega$$

and the fraction of the scattered radiation directed in the backwards direction per unit solid angle is given by $F(\pi) = B(\pi, h)/\beta(h)$. Then the transmittance due to aerosols, $T_A(h_1, h_2)$, between the heights h_1 and h_2 can be related to the integrated backscatter function by

$$-\ln [T_A(h_1, h_2)] = \tau_A(h_1, h_2) = \int_{h_1}^{h_2} \beta_A(h) dh \\ = F_A^{-1}(\pi) \int_{h_1}^{h_2} B_A(\pi, h) dh \quad (2)$$

For the stratosphere suggested values of $F_A(\pi)$ range between 0.013 (refs 5, 6) and 0.0199 (ref. 7).

The laser power $p(h)$ returned from a height h and detected by the lidar receiver is given by

$$p(h) = P_0 K T^2(0, h) [B_M(\pi, h) + B_A(\pi, h)] / h^2$$

where P_0 is the power transmitted, K is a system constant which includes the geometry and efficiency of the lidar optics and electronics, and $B_M(\pi, h)$ and $B_A(\pi, h)$ are the volume backscattering functions for air molecules and aerosols at the height h . Rearranging gives

$$B_A(\pi, h) = p(h) h^2 / \{ P_0 K T_M^2(0, h) \cdot T_0^2(0, h) \cdot T_A^2(0, h) \} \\ - B_M(\pi, h) \quad (3)$$

The solution of equations (2) and (3) for B_A and T_A is accomplished by an iterative technique developed by Elterman⁸. At the height or heights where the total scattering is a minimum the aerosol scattering is assumed to be zero, thereby normalising the lidar signal to the known molecular atmosphere at these heights. This procedure is justified by the detection of regions of very low aerosol content by direct sampling techniques. Values of B_A and T_A are then determined for successive height increments by iteration of equations (2) and (3) at each level. The

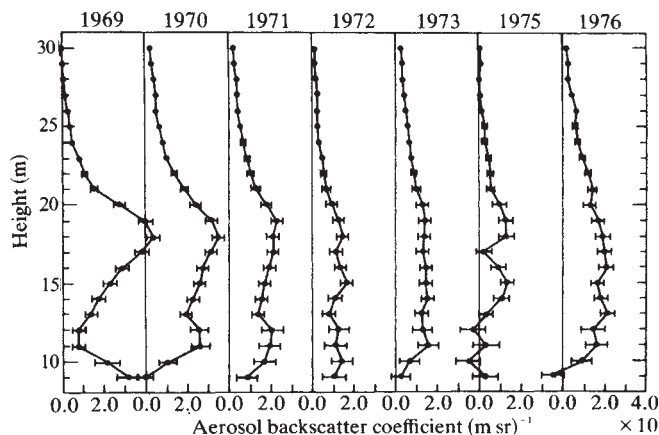


Fig. 1 Yearly means values of aerosol backscatter coefficient B_A . Error bars are ± 1 standard deviation.

constant $F_A(\pi)$ was taken as 0.0199; a choice of 0.013 would have changed the integrated backscatter function for the average of the data for 1969 by <2%. The molecular parameters B_M and T_M are calculated from the atmospheric density profile measured by radiosonde balloons launched from the Adelaide Airport, 7 km from the lidar site, on the night of the lidar measurements or on the following morning. The ozone transmittance was calculated from Eltermans tables¹.

The average yearly profiles of $B_A(\pi, h)$, shown in Fig. 1, show how the values of this function have varied with height and time. Error bars represent one standard deviation and include the statistical error in the signal photoelectron count rate and a 1% error⁹ in the radiosonde-measured density profile. Note that although each nightly profile was normalised to the molecular profile in one or more regions, the average profiles for some years show no region where the aerosol contribution to scattering is consistently zero. Failure of the ruby laser allowed only one observation between December 1973 and February 1976.

By integrating curves similar to Fig. 1 over the height range 10–30 km, the vertically integrated aerosol backscatter function^{6,10} is formed; the average monthly values are plotted in Fig. 2. The aerosol optical thickness is indicated by the scales on the right. Scale A refers to a value of $F_A(\pi)$ of 0.0199 and scale B to value of 0.013.

A maximum in aerosol optical thickness occurred in late 1969 followed by a decline thereafter until 1976. The occurrence of the peak value is related to the eruption of the Fernandina volcano in June 1968 and its timing is possibly a result of the gas-to-particle conversion rate¹¹ and the stratospheric meridional transport rates. There is a suggestion of an annual

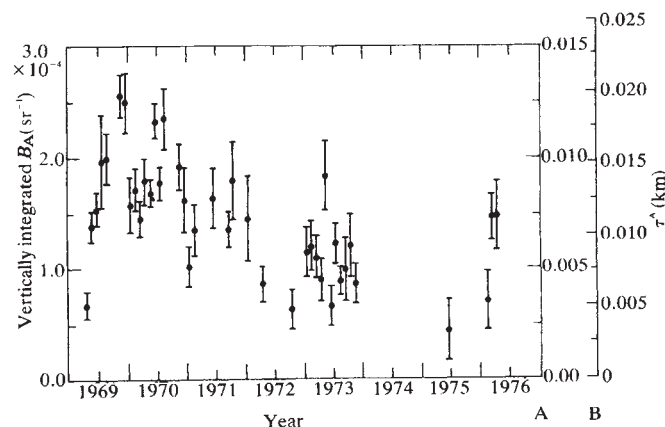


Fig. 2 Monthly mean values of vertically integrated backscatter coefficient (10–30 km), and corresponding values of aerosol optical thickness assuming values of $F_A(\pi) = 0.0199$ (scale A), and 0.013 (scale B).

variation in 1969 and 1970 with lower values early in the year. The abnormally high value in May 1973 and low value in June 1975 are each the result of observations on a single night and may not truly represent the average conditions in these months.

The peak values of aerosol optical thickness range between 0.013 and 0.020 (depending on the assumed value of F_A). These are considerably less than the values for 700 nm determined by Elterman from searchlight measurements in 1964 and 1965¹ (0.027) and are also less than his value for 1970¹². The average Adelaide value for 1973 is between 0.005 and 0.007. This is higher than the lidar-derived value of 0.004 obtained over California¹⁰ for the same period, but much lower than Elterman's⁸ value of 0.014 for the 'normal' stratosphere (when converted to 694 nm). The average of values for early 1976, 0.006–0.009 is slightly higher than the 1973 average and may indicate the presence of some residual dust from the eruption in October 1974 of Volcán de Feugo in Guatemala.

To obtain a reliable estimate of the minimum value optical thickness for the period of observation, a weighted average was calculated from those eight monthly values which were not statistically distinguishable from the lowest value. A value of $(7.4 \pm 0.2) \times 10^{-5}$ per steradian was obtained for the integrated backscatter function, which corresponds to an optical thickness of between 0.004 and 0.006. Thus the extreme values of aerosol optical thickness varied by a factor of at least three during the period 1969–76.

To relate the aerosol optical thickness for the lidar wavelength of 694 nm to the optical thickness for the solar spectrum, the extinction due to scattering was calculated for the two spectral distributions using the stratospheric aerosol size distribution given by Bigg¹³. The extinction of solar radiation is 87% of that for monochromatic radiation at 694 nm. Thus during the period 1969–76, the optical thickness of stratospheric aerosols for solar radiation varied from about 0.018 to 0.004.

An estimation of the reduction in the ground level solar flux at Adelaide during 1969–76 due to stratospheric aerosols can be obtained from the analysis of Cadle and Grams¹⁴. The energy lost due to upward scattering from a solar beam incident at a zenith angle of 60° varied between 0.5 and 0.15%.

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1. Elterman, L. AFCRL-68-0153, *Envir. Res. Pap.* No. 285 (1968).
2. Russell, P. B., Viezee, W. & Hake, R. D. Jr *Semi-Annual Report, SRI Project 2217* (1973).
3. Bartusek, K., Gambling, D. J. & Elford, W. G. *J. atmos. terr. Phys.* **32**, 1535–1544 (1970).
4. McCartney, E. J. *Optics of the Atmosphere* (Wiley, New York, 1976).
5. Deirmendjian, D. *Electromagnetic Scattering on Spherical Polydispersions* (Elsevier, New York, 1969).
6. Russell, P. B., Hake, R. D. Jr. & Viezee, W. *J. atmos. Sci.* **34**, 163–177 (1977).
7. Deirmendjian, D. *J. geophys. Res.* **70**, 743–745 (1965).
8. Elterman, L. *Appl. Opt.* **5**, 1769–1776 (1966).
9. Lenhard, R. W. *Bull. Am. met. Soc.* **54**, 691–693 (1973).
10. Russell, P. B., Viezee, W., Hake, R. D., Jr & Collis, R. T. H. *Q. J. R. met. Soc.* **102**, 675–696 (1976).
11. Castleman, A. W. Jr., Munkelwitz, H. R. & Manowitz, B. *Tellus* **26**, 222–234 (1974).
12. Elterman, L., Toolin, R. B. & Essex, J. D. *Appl. Opt.* **12**, 330–337 (1973).
13. Bigg, E. K. *J. atmos. Sci.* **33**, 1080–1086 (1976).
14. Cadle, R. D. & Grams, G. W. *Rev. Geophys. Space Phys.* **13**, 475–501 (1978).

Anomalous results for tidal flow through the Pentland Firth

THE flow of water across the vertical component of the Earth's magnetic field induces a potential gradient in the water; measurements of the difference in voltage between the opposite sides of a tidal channel provide an indication of the magnitude of the tidal flow. Longuet-Higgins¹ derived the relationship between the potential gradient and the tidal flow for the case of

uniform flow in a channel of semi-elliptic cross-section. By recording the difference in potential between the ends of submarine telephone cables valuable measurements have been obtained of flow through the Dover Strait^{2,3} and through various channels in the Irish Sea^{4,5}. However, recent cable measurements for tidal flow through the Pentland Firth are shown here to include anomalous results.

The study of the movement and dispersal of certain water masses over periods of years and, consequently, over large spatial zones is of increasing interest in connection with studies of dispersal of radioactive waste or fisheries investigations. Cable measurements are of particular value in such studies as they provide, at minimal cost, a continuous long-term record of the integrated flow across a particular section⁶. However, in recent years many of these cables have either been abandoned or replaced by 'repeated' cables. In the latter, power is fed along the cables to amplify the communications signal at repeated intervals and this tends to distort the measurement of the flow-induced voltage⁷.

Unexpected results found from recording on cables crossing the Pentland Firth (Fig. 1) are particularly interesting. Two cables, running along closely parallel paths, link the Orkneys with the Scottish mainland. Recordings of voltage were made on these cables from February to October 1976, coinciding with the JONSDAP '76 oceanographic exercise in the North Sea. The measurements coming respectively from the two cables were very similar so only the results from cable 2 are presented. Tidal analyses of the recordings were made by dividing the data into six discrete sets each lasting 29 d. Despite the high noise level, the analyses produced consistent results for the principal harmonic constituents (Table 1). However, an examination of the propagation of the M_2 tidal constituent shows that the phase of this constituent calculated from the cable data (that is 18° for flow measured positive towards the east) does not accord with the general pattern of flow as deduced from other data sources (Fig. 1).

Values for the phase of the M_2 constituent of surface currents were calculated from the Admiralty Tidal Stream Atlas. Figure 1 shows values of 271° and 256° obtained for positions in the Firth and a value of 235° at a position slightly east of the Firth. As part of JONSDAP '76 current meter measurements were made at position 53 (58°37' N, 2°25' W). Tidal analyses of these measurements produced an M_2 value of 302° for flow along the major axis of the ellipse aligned 10°W of S. The phase of the vertical tide in this region is indicated by the co-phase lines in Fig. 1 and also by the values from the coastal gauges. Over the region in which the phase of the currents varies from 271° to 302°, the phase of the vertical tide varies from about 250° to

Table 1 Tidal analyses of voltages recorded on the Pentland Firth telephone cable

	Amplitude				Phase			
	1976		1978		1976		1978	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
O1	20	22	29	31	23°	24°	7°	19°
K1	44	47	82	20	172°	22°	193°	28°
μ_2	45	24	50	6	251°	11°	254°	7°
N2	52	10	56	21	325°	7°	339°	6°
M2	231	3	236	3	18°	3°	19°	1°
L2	17	42	18	75	111°	21°	148°	51°
S2	105	11	111	20	61°	8°	66°	5°
M4	10	47	6	22	203°	14°	190°	92°
M6	6	57	12	16	69°	44°	66°	25°
	mV	%*	mV	%†				

* Standard deviation of results from six separate monthly analyses expressed as a percentage of the mean value of the constituent.

† Standard deviation of results from three separate monthly analyses.

320°. Thus maximum flows apparently occur within 1 h of tidal high water throughout this region. Therefore the anticipated M_2 phase for flow across the cable section would be in the range 280°–340° compared with the recorded value of 18°.

An examination of the non-tidal (or residual) component from the cable signal shows a reasonable correlation ($r = 0.71$) between the easterly flows through the Pentland Firth and the southerly flows measured by current meters at the nearby position 53. There was no indication of either a significant wind-driven component or a seasonally varying flow component in the residual signal.

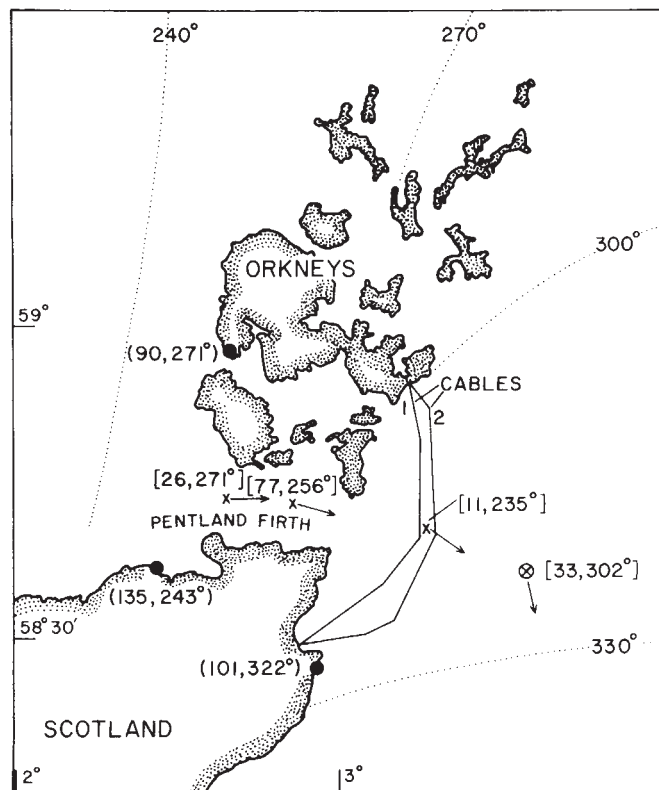


Fig. 1 Propagation of the M_2 tidal constituent in the region of the Pentland Firth. ●, Tidal elevation data in cm; x, tidal stream data in cm s^{-1} (from Admiralty Tidal Stream Atlas); ⊗, current meter data from JONSDAP '76; co-phase lines shown dotted.

One explanation for the anomalous M_2 phase follows from the work of Robinson⁸ who showed that cables respond in a complex fashion to flow over a wide area surrounding the termination points of the cable. He also showed that non-uniformity in sea-bed conductivity can complicate the general integral. In this region, the variability in both the coastline and the tidal stream patterns tends to invalidate the application of simplified formulae of the kind derived by Longuet-Higgins. In addition, the northerly latitude and part east-west alignment of the cable increase the noise level on the cable⁹. The question also arose as to whether any error had been introduced either in the instrumentation or in the data processing.

To establish the validity of these results the recordings were repeated over a 3-month period, July–September 1978. One of the cables had been severed in the interim period and so measurements could only be made on cable 2. To avoid repeating possible errors from the earlier experiment, the recording equipment was installed by a scientist not involved in the earlier recordings. Also, the programmes used to convert the original data into hourly values for subsequent analyses were rewritten. The data were sub-divided into three discrete sets, each of 29 d

duration, for the purpose of tidal analyses. The results, shown in Table 1, closely agree with the values obtained from the 1976 recordings. Thus the voltage recordings from the Pentland Firth submarine cables seem to provide a useful indication of residual flow through the channel but produce an anomalous M_2 tidal signal.

R. I. R. Palin and A. J. Harrison were responsible for the instrumentation and data collection, their assistance in data translation also contributed significantly to this study. This work was funded by a Consortium consisting of the NERC, the Ministry of Agriculture Fisheries and food, and the Departments of Energy, and Industry.

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1. Longuet-Higgins, M. S. *Mon. Not. R. astr. Soc. geophys. Suppl.* **5**, 285–307 (1949).
2. Bowden, K. F. *Phil. Trans. R. Soc. A* **248**, 517–551 (1956).
3. Alcock, G. A. & Cartwright, D. E. *A Voyage of Discovery, George Deacon 70th Anniversary Vol* (ed Angel, M. V.) 341–365 (Pergamon, Oxford, 1978).
4. Hughes, P. *Limnol. Oceanogr.* **14**, 269–278 (1969).
5. Prandle, D. *Geophys. J. R. astr. Soc.* **45**, 437–442 (1976).
6. Prandle, D. *J. mar. biol. Ass. U.K.* **58**, 965–973 (1978).
7. Alcock, G. A., Harrison, A. J. & Palin, R. I. R. *Inst. Oceanogr. Sci. Rep.* No. 62 (1978).
8. Robinson, I. S. *Phil. Trans. R. Soc. A* **280**, 355–396 (1976).
9. Axe, G. A. *P.O. elect. Engrs' J.* **61**, 37–43 (1968).

A simple approach for identifying and measuring acidification of freshwater

ACIDIFICATION of lakes and rivers accompanied by the loss of fish populations is a problem in Scandinavia^{1–4}, northeastern USA^{5,6}, and southeastern Ontario, Canada^{7,8}. These areas have granitic or other siliceous bedrock types, thin and patchy podsol soils, and extremely soft and poorly buffered surface waters, and receive precipitation which is decidedly acidic (volume-weighted average pH 4.0–4.6). Sulphate, whose principal source is precipitation, is usually the major anion in these acidified waters. In similar areas such as northern Scandinavia and northwestern Ontario, in which precipitation is not acidic (pH > 5.0) such oligotrophic pristine softwaters generally have pH levels > 5.5, and bicarbonate is the major anion^{9–11}. Acidification of such waters entails a decrease in the bicarbonate buffer (alkalinity) with only minor decreases in pH, and then after exhaustion of the bicarbonate an increase in acidity to pH levels well below 5.0. Because of the severe damage to fish populations, and other biological consequences, the decreases in pH that characterise the later stages of the acidification process are of major concern. We need, therefore, a simple 'early warning' indicator that identifies lakes and rivers which are undergoing the first stage of acidification, the loss of alkalinity, but which have not yet reached the stage of marked pH decreases. Furthermore, we need quantitative estimates of the degree of acidification. We show here that excess sulphate concentrations, or current calcium concentrations and alkalinities can provide such an estimate.

If bicarbonate is still present, simply measuring alkalinity will not reveal whether or not acidification has occurred unless pre-acidification alkalinity data are available for comparison. Unfortunately, few such data exist. However, in unacidified waters bicarbonate alkalinity is present in approximately equivalent concentrations as the sum of calcium and magnesium (less that of sea-spray origin). As the ratio Ca/Mg is also relatively constant, and pH is closely related to alkalinity and acidity, plots of pH and calcium should distinguish between

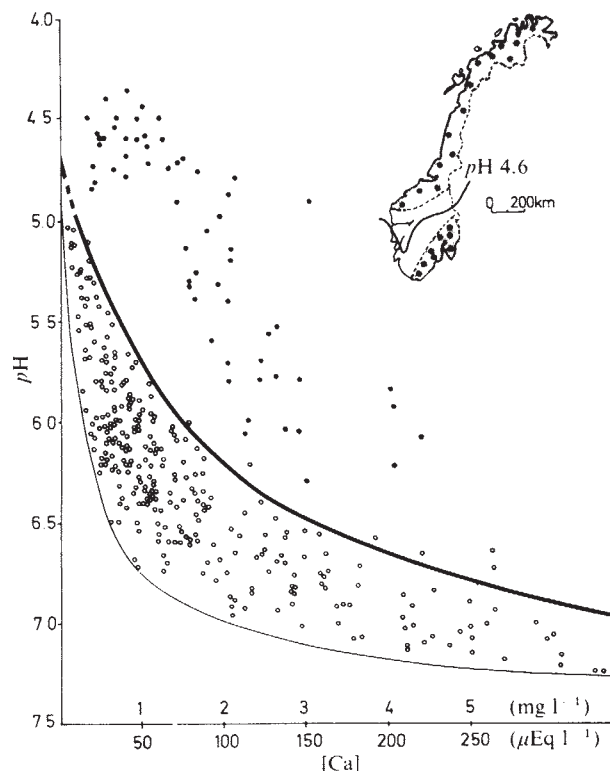


Fig. 1 pH and calcium concentrations in lakes in northern and northwestern Norway sampled as part of the regional survey of 1975, in lakes in northwestern Norway sampled in 1977 (○) and in lakes in southernmost and southeastern Norway sampled in 1974 (●). Southern Norway receives highly acid precipitation (pH 4.2–4.5) and a large number of lakes have lost their fish populations due to high acidity^{2,4}. Inset shows areas in which these lakes are located. Areas south of isoline receive precipitation more acid than pH 4.6.

acidified and unacidified waters. This is the case for small Norwegian lakes sampled in 1975 and 1977: most of the lakes in northern and northwestern Norway fall below an empirically drawn curve, whereas lakes in southernmost and southeastern Norway lie above this curve (Fig. 1).

The curve also separates lakes on the west coast of Sweden where pH in precipitation is 4.2–4.4 from lakes in west-central Sweden (W. Dickson, personal communication) where pH in precipitation is less acid (Fig. 2). Furthermore, it also works for North America. Lakes in the Experimental Lakes Area (ELA), northwestern Ontario¹¹, and in the Sierra Nevada, California¹² (Fig. 3), areas not receiving highly acidic precipitation, fall below the curve, whereas waters in acidified areas such as the Adirondack Mountains, New York⁶, Hubbard Brook, New Hampshire¹³, and Sudbury, Ontario⁷, fall above the curve.

Figures 1–3 clearly show that the empirical curve distinguishes between lakes situated in non-acidified and acidified areas in Scandinavia and North America. This acidification indicator is particularly useful in that it identifies lakes that have lost some alkalinity but in which pH levels are still high enough for fish populations and other components of the biota to be apparently intact and normal; these waters may be threatened by future inputs of acid components. Thus an 'early warning' is given.

This indicator depends on the observation that in oligotrophic pristine freshwaters calcium is accompanied by a proportional amount of bicarbonate. In such areas, one finds waters in which this is not the case, for example waters containing high concentrations of natural organic acids (highly coloured waters), or waters in areas where the geological environment is such that calcium and bicarbonate are not the major cation and anion, respectively. When applied to data from such waters, the indicator implies an apparent acidification where none has occurred.

The acidification of a lake is the loss in alkalinity that has taken place in the lake water. To quantify acidification we thus need to know the pre-acidification alkalinity and the present-day alkalinity (or acidity in case of acid lakes):

$$\text{Acidification} = \text{pre-acidification alkalinity} \\ - \text{present day alkalinity.}$$

As few historical alkalinity values are available for lakes that are acidified today, an estimate of the pre-acidification alkalinity is therefore needed. Present-day calcium concentrations can be used to estimate alkalinity because in regions not receiving acid precipitation softwater oligotrophic waters contain proportional concentrations of calcium and alkalinity. For example, calcium and alkalinity data from 59 lakes in northern and northwestern Norway gave a least-squares linear regression alkalinity ($\mu\text{Eq l}^{-1}$) = $-29 + 1.32 \text{ Ca } (\mu\text{Eq l}^{-1})$, $r = 0.92$. Similarly, data from 98 lakes in the ELA¹¹ gave alkalinity ($\mu\text{Eq l}^{-1}$) = $-32 + 1.42 \text{ Ca } (\mu\text{Eq l}^{-1})$, $r = 0.87$. (If alkalinity is determined by the standard acidimetric titration to pH 4.5, actual bicarbonate concentrations are overestimated by $32 \mu\text{Eq l}^{-1}$, the amount of free H^+ -ion in solution at pH 4.5. For these regressions the alkalinity data obtained by titration were corrected for this $32 \mu\text{Eq l}^{-1}$.)

These data suggest that the relationship between calcium and alkalinity in pristine softwater lakes is of a general nature. Thus pre-acidification alkalinities can be estimated from present calcium concentrations.

One factor must, however, be considered when estimating pre-acidification alkalinities from present-day calcium concentrations. There is evidence for an increase in the calcium concentrations in acidified areas due to increased chemical weathering and washout from the soil^{10,14,15}. Thus present-day calcium concentrations might overestimate the pre-acidification alkalinity. On the other hand, an increased washout of calcium from a lake catchment probably also results in an equivalent amount of alkalinity which is consumed during the acidification process.

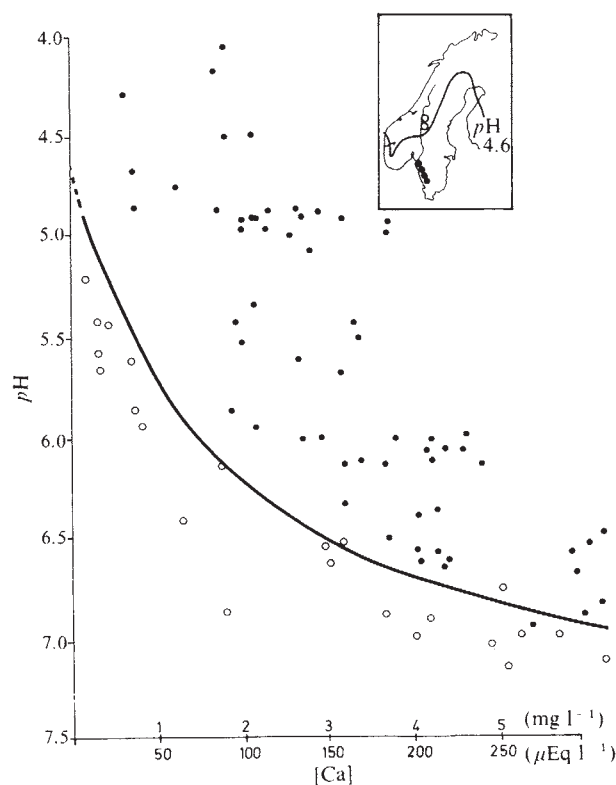


Fig. 2 pH and calcium concentration in lakes in southern (●) and west-central Sweden (○) (data from W. Dickson). Inset shows locations of lakes sampled. Areas south of isoline receive precipitation more acid than pH 4.6.

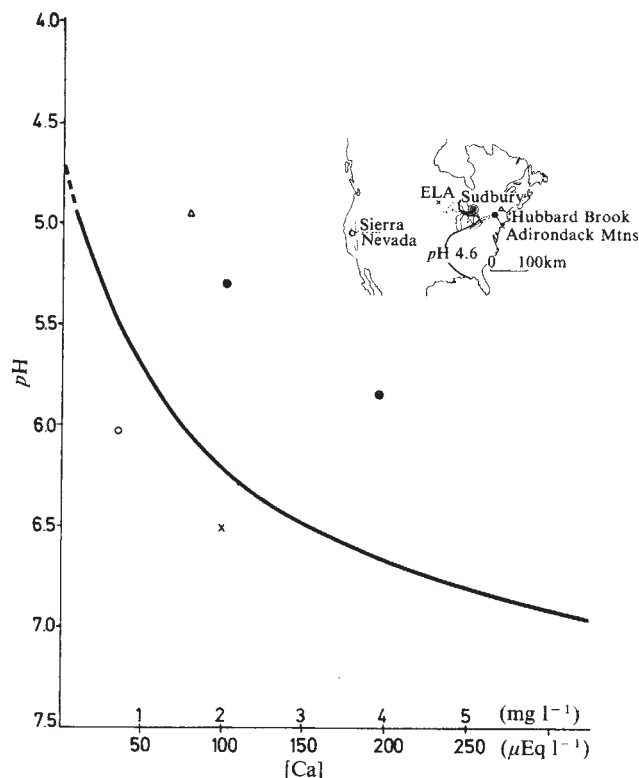


Fig. 3 Average pH and calcium concentrations in 170 lakes in the Sierra Nevada, California¹² (○), 109 lakes in the Experimental Lakes Area, northwestern Ontario¹¹ (×), 178 lakes in the vicinity of Sudbury, Ontario⁷ (⊗), 216 lakes in the Adirondack Mountains, New York (●) (Schofield, personal communication), and average for 11 yr at Hubbard Brook, New Hampshire¹³ (△). Waters in areas receiving acid precipitation lie well above the solid curve, whereas those in other areas lie below. Inset shows locations of these areas. Areas east of the isoline receive precipitation more acid than pH 4.6.

Regional surveys of lakes in southern Norway have been conducted annually since 1974 (ref. 16). Strong acid concentrations using the Gran technique¹⁷ were measured in the 1978 samples, and when combined with pH, calcium and sulphate data, estimation of the degree of acidification can be made for 46 lakes. The acidification estimated this way is closely related to the weighted average concentration of H^+ and sulphate in precipitation at each lake (Fig. 4a, b). These data indicate that significant acidification has occurred in areas receiving precipitation with volume-weighted average concentrations of H^+ above about $20\text{--}25\ \mu\text{Eq l}^{-1}$ (pH 4.7–4.6) and SO_4 concentrations above $1\ \text{mg l}^{-1}$ ($20\ \mu\text{Eq l}^{-1}$). This 'threshold' pH level of 4.6–4.7 was also determined by Wright¹⁸ in an analysis of historical pH data from 217 lakes in southern Norway and Sweden. Lakes in which pH had declined significantly during the past 20–25 yr are located exclusively in areas receiving precipitation more acid than about pH 4.6.

The estimated acidification of lakes in southern Norway is also strongly related to the concentrations of excess sulphate in the lake water, that is, sulphate in excess of that of marine origin as measured by chloride concentrations and the $SO_4:Cl$ ratio in seawater (Fig. 4c). This suggests that the bicarbonate lost in acidified lakes has been replaced by an equivalent amount of sulphate. The excess sulphate concentration in lakes less a 'general Northern Hemisphere' concentration then provides another measure of the acidification. Figure 4a, b indicates that about $10\text{--}20\ \mu\text{Eq l}^{-1}$ ($0.5\text{--}1.0\ \text{mg SO}_4\ \text{l}^{-1}$) is a reasonable estimate of the natural background concentration for lakes in southern Norway. Almer *et al.*¹⁰ suggest $20\text{--}60\ \mu\text{Eq l}^{-1}$ as a general background for Sweden.

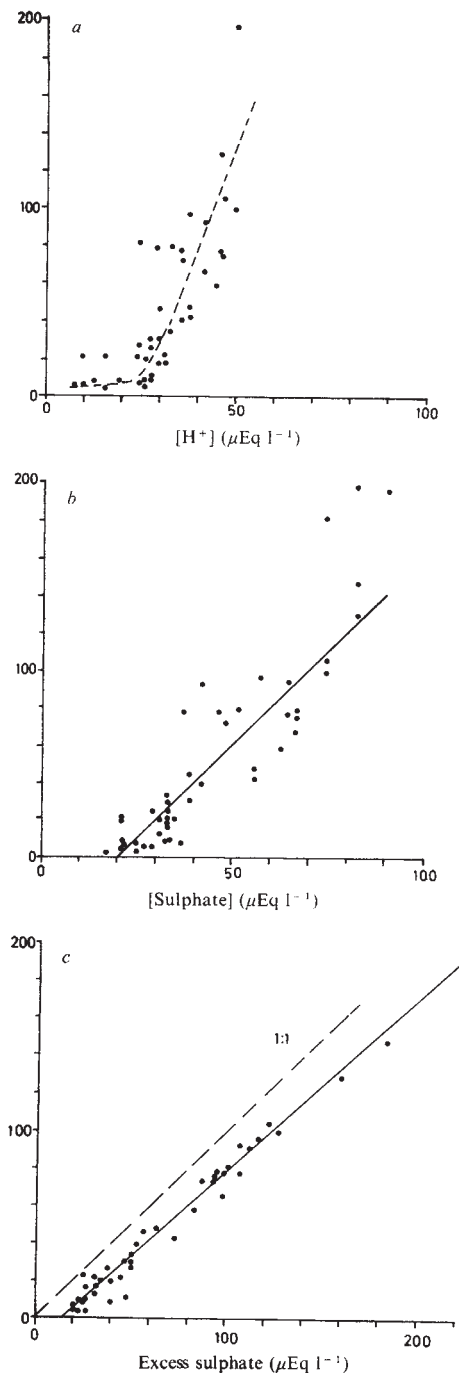


Fig. 4 Estimated acidification in 46 lakes in south Norway versus H^+ -concentration (a) and excess sulphate (b) in precipitation at each lake. Precipitation data were interpolated from data given by Dovland *et al.*¹⁹. c, Estimated acidification versus excess sulphate concentrations in 46 lakes. Least squares regression line: $y = -12 + 0.89x$ ($r = 0.99$).

Thus, in areas in which very little sulphur is supplied by weathering and where the ratio of non-marine calcium to magnesium is fairly constant, acidification of freshwaters can be estimated from either present-day excess sulphate concentrations or by present-day calcium concentrations and alkalinities.

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1. Gjessing, E. T., Henriksen, A., Johannessen, M. & Wright, R. F. *SNSF-proj. FR 6/76*, 64–85 (NISK, Ås, 1976).
2. Leivestad, H., Hendrey, G. R., Muniz, I. P. & Snekvik, E. *SNSF-proj. FR 6/76*, 86–111 (NISK, Ås, 1976).
3. Odén, S. *Water, Air, Soil Pollut.* **6**, 137–166 (1976).
4. Jensen, K. W. & Snekvik, E. *Ambio* **1**, 223–225 (1972).
5. Likens, G. E. *Chem. Engng News* **56**, 29–44 (1976).
6. Schofield, C. L. *Ambio* **5**, 228–230 (1976).
7. Conroy, N., Hawley, K., Keller, W. & LaFrance, L. *J. Great Lakes Res.* **2**, Suppl. 1, 146–165 (1976).
8. Beamish, R. J. & Harvey, H. H. *J. Fish. Res. Board Can.* **29**, 1131–1143 (1972).
9. Wright, R. F. & Henriksen, A. *Limnol. Oceanogr.* **23**, 487–498 (1978).
10. Almer, B., Dickson, W., Ekström, C. & Hörnström, E. in *Sulfur in the Environment*, part 2, (ed. Nriagu, J. O.) 272–311 (Wiley, New York, 1978).
11. Beamish, R. J., Blouw, L. M. & McFarlane, G. A. *Tech. Rep. No. 607* (Dept of the Environment, Fisheries and Marine Services, Canada, 1976).
12. Bradford, G. R. Bair, F. L. & Hunsaker, V. *Limnol. Oceanogr.* **13**, 526–530 (1968).
13. Likens, G. E., Bormann, F. H., Pierce, R. S., Eaton, J. S. & Johnson, N. M. *Biogeochemistry of a Forested Ecosystem* (Springer, Berlin, 1977).
14. Henriksen, A. *Vann* **7**, 65–73 (1972).
15. Dillon, P. J., Yan, N. D., Scheider, W. A. & Conroy, N. *Archs Hydrobiol. Ergeb. Limnol.* (in the press).
16. Wright, R. F. *et al. SNSF-proj. IR 33/77* (NISK, Ås, 1977).
17. Stumm, W. A. & Morgan, J. J. *Aquatic Chemistry* (Wiley, New York, 1970).
18. Wright, R. F. *SNSF-proj. TN 34/77* (NISK, Ås, 1977).
19. Dovland, H., Joranger, E. & Semb, A. *SNSF-proj. FR 6/76*, 15/35 (NISK, Ås, 1976).

Palaeogene komatiites from Gorgona Island

UNMETAMORPHOSED mafic and ultramafic rocks overlain by Neogene oceanic sediments are exposed on Gorgona Island which is a remnant of the Coastal Cordillera along the Pacific margin of Colombia¹. Gorgona is one of the rare places in the world where young pyroxenitic komatiites exhibiting typical quenched spinifex textures (Fig. 1) occur. The spinifex rocks form lenticular bodies (? lava flows) within cumulate ultramafics and troctolitic gabbros and are closely associated with diabases and tholeiitic pillow basalts¹. We show here that in textures, rock and mineral chemistry, the Gorgona spinifex rocks are almost identical with the Archean peridotitic to pyroxenitic komatiites from Munro Township (North-east Ontario)². For this reason the term komatiites is used for the Palaeogene rocks, although one could call them high Mg-tholeiites.

Mineral composition: quenched coarse olivine blades up to 5 cm long and 1 mm thick dominate the spinifex texture. The blades are Fo_{90–91} with narrow edges of Fo_{85–86}. Clinopyroxenes and plagioclases are restricted to the interstitial groundmass (Fig. 1). High-aluminium calcic pyroxenes exhibit coarse skeletal crystals; whereas low-aluminium calcic pyroxenes, unzoned plagioclase laths (An_{75–81}) and devitrified and chloritised glass form fine-grained, arborescent crystal aggregates. Chromite occurs mostly in idiomorphic grains.



Fig. 1 Photomicrograph of the spinifex-textured komatiite from Gorgona Island. Weakly serpentinized skeletal plates of olivine are randomly scattered around a groundmass consisting of quenched clinopyroxene aggregates, few plagioclases and devitrified glass.

Rock composition: the major and trace elements have been mainly determined by X-ray fluorescence methods³, the rare earth elements by instrumental neutron activation analysis (INAA in Karlsruhe) (see Table 1).

Outstanding chemical features of these rocks are the high MgO, chromium and nickel contents relative to ocean floor basalts. The olivine compositions seem to be compatible with crystallisation from a liquid with magnesium-values corresponding to the bulk rock composition, giving K_D values (ol/rock)⁴ of about 0.3. We think that the unusual chemical

Table 1 Rock composition of komatiites from Gorgona Island

	Average of four determinations wt%	Calculated anhydrous wt%	Trace elements (p.p.m.)					
SiO ₂	44.2	46.2	Nb	<1	La	1.1		
TiO ₂	0.66	0.69	Zr	30	Ce	~1 ± 1		
Al ₂ O ₃	12.0	12.6	Y	14	Nd	~1 ± 1		
Fe ₂ O ₃	3.2	1.3	Sr	58	Sm	1.7		
FeO	8.2	10.3	Rb	<1	Eu	0.75		
MnO	0.18	0.19	U	<1	Gd	3.4		
MgO	15.9	16.6	Th	<4	Tb	0.49		
CaO	10.1	10.5	Pb	<6	Ho	0.89		
Na ₂ O	1.13	1.18	Ga	15	Tm	0.27		
K ₂ O	0.02	0.02	Zn	57	Yb	1.5		
P ₂ O ₅	0.06	0.06	Cu	105	Lu	0.17		
Cr ₂ O ₃	0.18	0.19	Ni	718	Ba	<1		
NiO	0.09	0.09	Co	93	Sc	31		
H ₂ O	4.0	—	Cr	1250	S	109		
Total	100.0	100.0	V	256				

Mg-value:

$$\frac{100 \text{ Mg}}{\text{Mg} + \text{Fe}^{++}} = 77.6$$

FeO:Fe₂O₃ = 9:1

$$= 74.2$$

$$K_D = \frac{X_{\text{FeO}}^{\text{ol}} \cdot X_{\text{MgO}}^{\text{rock}}}{X_{\text{FeO}}^{\text{rock}} \cdot X_{\text{MgO}}^{\text{ol}}}$$

composition of these rapidly chilled extrusive rocks within the close neighbourhood of oceanic crust throws new light on the petrological models of generation of ocean floor basalts; by far the most common rock type on Earth. There are several possibilities for the origin of ocean floor basalts: do they represent relatively unmodified primary magmas produced by partial melting from a hercynite type source rock⁴, did they originate from other ultramafic mantle material followed by crystal fractionation⁵ or could magma mixing of mantle-derived primary magmas together with highly evolved differentiation products along with crystal fractionation have played an important part^{6,7}?

We believe that the Gorgona komatiites represent the least modified liquid generated by partial melting of the upper mantle. They have certain chemical characteristics similar to the melt inclusions in olivine and plagioclase from Mid-Atlantic ridge basalts⁷ (DSDP, Legs 45 and 46, Sites 395 and 396). These include low TiO₂ and Na₂O contents, and high magnesium-values. However, the Gorgona komatiites have higher MgO and nickel contents and are closer to the primary magma, proposed on the basis of experimental evidence⁶, than are the most 'primitive' ocean-floor basalts described to date. Green *et al.*'s primary magma which could have segregated from residual harzburgite at ~20 kbar, 1,430 °C, was experimentally derived from basalt glass (south Mid-Atlantic Ridge, DSDP Leg 3, Site 18, sample 3-18-7-1)⁸ which contains microphenocrysts of olivine (Fo_{89.5}) and plagioclase (An₇₉). This glass also shows very similar rare earth patterns to the Gorgona komatiites. Both are strongly depleted in light rare-earth elements and have low concentrations of large-ion lithophile elements. These aspects will be discussed in more detail elsewhere⁹. Geochemical investigations of the surrounding mafic rocks¹⁰ show that Gorgona Island consists mainly of normal fractionated olivine tholeiites, diabases, troctolites and gabbros, which could be explained by the existence of a shallow magma chamber^{6,7}. The question still remains of whether Gorgona Island represents an uplifted part of a large Pacific oceanic crust or a smaller marginal basin.

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1. Gansser, A. *Schweiz. Mineral. Petrogr. Mitt.* **30**, 219–237 (1950).
2. Arndt, N. T., Nalder, A. J. & Pyke, D. R. *J. Petrol.* **18**, 319–369 (1977).
3. Nisbet, E. G., Dietrich, V. J. & Esenwein, A. *Fortsch. Miner.* (submitted).
4. Bender, J. F., Hodges, F. N. & Bence, A. E. *Earth planet. Sci. Lett.* **41**, 277–302 (1978).
5. Kay, R., Hubbard, N. J. & Gast, P. W. *J. geophys. Res.* **75**, 1585–1613 (1970).
6. Green, D. H., Hibberson, W. O. & Jaques, A. L. in *The Earth: Its Origin, Structure and Evolution* (ed. McElhinny, M. W., Academic, London, 1978).
7. Rhodes, J. M., Dungan, M. A., Blanchard, D. P. & Long, P. E. *Tectonophysics* (in the press).
8. Frey, F. A., Bryan, W. B. & Thompson, G. *J. geophys. Res.* **79**, 5507–5527 (1974).
9. Cameron, W. E., Dietrich, V. J. & Gansser, A. *Contr. Miner. Petrol.* (submitted).
10. Dietrich, V. J., Gansser, A. & Sommerauer, J. *Schweiz. Mineral. Petrogr. Mitt.* (submitted).

Gametogenesis and calcification of planktonic Foraminifera

THE processes of gametogenesis and development in benthic Foraminifera are well known^{1,2}; however, only recently has there been substantial success in the culture of planktonic species³. As part of an investigation of planktonic life cycles, we have maintained in laboratory culture mature foraminifers of *Hastigerina pelagica* and *Orbulina universa* for up to 68 d with more than 80% of the individuals producing gametes. Juvenile stages were subsequently cultured up to initial calcification and regular chamber formation. Although other workers^{4–6} have cultured adults and induced gametogenesis, this is the first known success in the maintenance of gametes and the culture of second generation Foraminifera.

Live, adult, planktonic foraminifers were obtained from pelagic waters off Bermuda by hand capture according to a technique of Bé *et al.*³. Although this method limits specimens to those visible to a diver in 3–20 m of water, it yields healthy individuals undamaged by net tows or conventional samplers. Each organism was isolated in 500–700 ml of filtered natural seawater and aerated lightly. Normal ambient temperatures (19–23 °C) and lighting (12 h, 40–50 foot candles, fluorescent source) were used. To maintain axenic cultures, the seawater was filtered through successive 0.45-µm and 0.1-µm Millipore filters before the introduction of the foraminifers and repeated periodically to remove accumulated by-products. Adult specimens were removed to separate Petri dishes for biweekly feedings of *Thalassiosira fluviatilis* and nauplii of *Artemia* sp. grown in sterile conditions. *H. pelagica* readily accepted both, suggesting that it is an indiscriminate omnivore in nature. *O. universa* did not accept crustaceans, although carnivorous diets have been reported for it as well as for other spinose species^{1,3,5,7}. In any case, our results indicate that planktonic Foraminifera can be maintained readily on an exclusively carnivorous diet although healthiest individuals result from a combined diet of phytoplankton and live Crustacea.

Based on observations of 29 *H. pelagica* and two *O. universa*, gametogenesis can be said to be a predictable development

preceded by several characteristic morphological changes. Between 24 and 36 h before gamete release, the normally expanded calymma decreases until no bubbles or only a single bubble layer is visible around the chambers. Simultaneously, all pseudopodial filaments are retracted into the calcareous test and the main body of cytoplasm constricts into the inner chambers until the last and typically largest chamber is evacuated. The cytoplasm progressively darkens from pale orange or tan to deep orange or golden brown. Previous workers have attributed intense orange-red hues to redistributed lipid storage products⁶; however, in our specimens, orange pigmentation is a dietary phenomenon. While cytoplasmic coloration intensified in all specimens, only those fed high proportions of *Artemia* were deep orange. Others fed exclusively *Thalassiosira* tended to brown and, in one case of gametogenesis within 5 d of capture, to dark grey or black. The latter coloration may be due to local pelagic species of Crustacea in Bermuda, including the copepods *Candacia ethiopica*, *Euchaeta marina* and *Harpacticus* sp., which have black pigments and are sufficiently large to be a feasible food source for these foraminifers.

In *O. universa*, cytoplasmic regression and loss of the calymma coincided with a loss of all major spines. In contrast, in

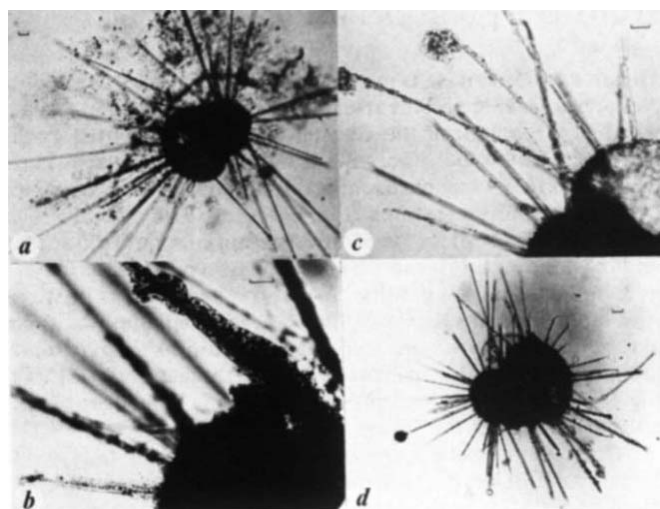


Fig. 1 Light micrographs of major gametogenic stages of *H. pelagica*. Scale bars, 25 µm. *a*, Onset of explosive release. Gametes and cellular residue are emitted only from major apertures and are densest near remains of terminal chamber. *b*, Masses of gametes flowing along spines in cytoplasmic strands during early to middle stages of gradual release. A few remnants of the parent bubble capsule are evident. *c*, Gamete clumps encased in hyaline material on spines near aperture. Unoccupied spines are dissolving inside a sheath formed by the parent organism. *d*, Terminal stages 10–12 h after release began. Few gametes remain in vicinity of parent test; most spines are resorbed leaving only membranous sheath or base.

no *H. pelagica* were all spines shed and the degree of spine loss or disintegration varied between individuals; remaining spines occasionally served to transport gametes to the periphery (Figs 1b, 3b). Spines in *H. pelagica* may be lost by resorption of their external bases⁵, by release of the spine from its base, or by progressive disintegration of the spine from its peripheral end (Fig. 1c). In five *H. pelagica*, spines began disintegration after onset of gamete release and were effectively dissolved in 4–6 h. The process is assumed to be autolytic because surface seawater is supersaturated in CaCO₃ and would not induce rapid dissolution. Most spines were encased in a residual, membranous sheath which, as the spine disintegrated, thinned, curving at the peripheral end. More fibril spines were often held in place by secondary mucoid strands. Experiments now in progress

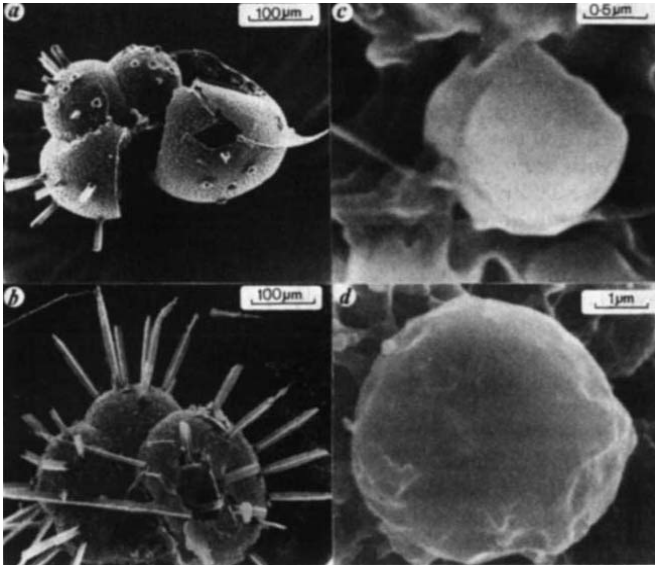


Fig. 2 *a*, Glutaraldehyde-fixed adult at 8-h stage of gamete release. Remains of organelles and developing gametes are found near lip of major aperture. *b*, Post-gametogenic test left 3 weeks in culture. Spines show dissolution pattern. *c*, Biflagellated gamete of *H. pelagica*, fixed 12–15 h after release. The flagella measure 7–10 μm and 12–15 μm respectively. *d*, Initial chamber (proloculus) formed 28 h after gamete release.

indicate that patterns of disintegration within the sheath resemble those of empty foraminiferan tests; however, the triradiate spines of vacated tests require up to 6 weeks to produce the same degree of etching as can be produced in 4 h in a gametogenic individual (Fig. 2*b*). The fragility of *Hastigerina* tests was noted by Berger⁸ and a similar spicule dissolution phenomenon shown for other pelagic protozoans⁹. These factors along with limited latitudinal distribution, may explain the relative scarcity of *Hastigerina* in marine sediments.

The actual release of gametes may be explosive or gradual and occurs 2–3 h after the test surface becomes rugose and the cytoplasm dense and granular. In dehiscence, cytoplasm fills the final chambers and gametes are discharged with protoplasm.

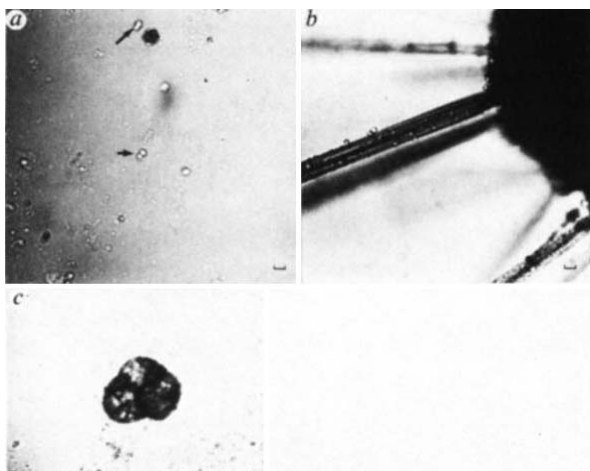


Fig. 3 *a*, Syngamy found in culture water 36 h after release, and *b*, on spines of parent test. A pair in which the process is nearly complete can be seen at top centre of *a*. Scale bar, 5 μm . *c*, Three-chambered *H. pelagica* produced in culture within 15 d of gametogenesis. Chambers shown range 27–40 μm in diameter.

The final chamber, formed 4–10 d before the onset of gametogenesis in 20% of our cultures, is lost entirely or part of its outer wall destroyed by forcible expulsion of its contents (Fig. 2*a*). Cytoplasmic residue is later released in a characteristic, streaming pattern (Fig. 1*a*), as reported for *Globigerinella aequilateralis*⁵, and closely resembling phases described for *Elphidium crispum*¹. In gradual release, intensification of the cytoplasm is followed by gametes being extruded along the spines. In spite of rapid flagellar movement, the gametes are normally held in a line by a mucoid coat similar to that covering dissolving spines (Fig. 1*b*). Substantial clumps form at the spine tips within 2 h of the onset of release (Fig. 1*c*) and the flow may continue for 8–10 h. Most gametes, however, reach the periphery and separate from the clumps within 3 h. At this stage, the gametes are dark, ovoid bodies, 2–5 μm long, and have two distinct, unequal flagella with a common base (Fig. 2*c*). The gametes can move just after release although rapid motion does not begin until they are 50–100 μm from the surface of the parent test. Movement is typically a stationary oscillation with rapid flagellar beating or rotation as though due to spinning flagella. Flagellar movement was too rapid to be clearly discerned during swimming. Mobile flagellated gametes survived in culture up to 12 d although most of them calcified or died within 5 d.

Syngamy was rare, observed among gametes of only four *H. pelagica*. No single parameter was common to the cultures in which copulation took place, nor were there apparent morphological differences from other gametes. An estimated maximum of 10% of the population formed syngamous pairs, and frequency of copulation was not increased by combining gametes from two specimens reproducing within the same 36 h. The pattern of copulation was invariable, one gamete remaining relatively still while a second moved rapidly around its periphery, periodically withdrew 10–15 μm , and then forcibly pushed against it. Contact generally occurred between the anti-flagellar ends of the gametes with adherence evident at the point of impact (Fig. 3). While thus attached, the two pairs of flagella straightened and beat rapidly, and the gamete pair whirled in the water. The more mobile of the two then broke off, withdrew, and repeated the battering process. Separation became progressively more difficult due to coalescence. This activity continued up to 30 min, ending in a single perceptible organism, about 5 μm in diameter, with two pairs of inactive flagella (Fig. 3*a*). Nuclear material could not be resolved in the living material, but a transfer or fusion is assumed.

Calcification to a single chamber, presumed a proloculus, took place as soon as 9 h after gamete release although most cultures did not show calcification for several days. Single-chambered organisms thus formed were smooth spheres, 8–20 μm across, with few or no apertures perceptible (Fig. 2*d*). The degree of calcification varied, with some individuals forming overlapping layers; however, that may have been a culture abnormality. In only one of our cultures have we found chamber formation beyond prolocula. Two polythalamic individuals were produced (Fig. 3*c*), each forming four chambers within 15 d of gametogenesis, the last chamber appearing within 2 d of the third. The organisms measured 65–80 μm in diameter with the largest chambers 35 and 40 μm in diameter. Both were planispiral with irregularly arranged pores about 1 μm wide and a single 10- μm aperture in the final chamber. No spines have been produced on either individual. None of our second generation specimens has matured beyond this stage and the main difficulties foreseen in further culturing are the provision of appropriate food sources and the formation of culture aberrants with a small sample size.

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1. Le Calvez, J. in *Traité de Zoologie*, tome 1, fasc. 2 (ed. Grassé, P-P) 149–265 (Masson, Paris, 1953).
2. Grell, K. G. *Protozoology* (Springer, New York, 1973).
3. Bé, A. W. H. *et al. Micropaleontology* **23**, 155–179 (1977).
4. Adshead, P. C. *Micropaleontology* **13**, 32–40 (1967).
5. Bé, A. W. H. & Anderson, O. R. *Science* **192**, 890–892 (1976).
6. Spindler, M., Anderson, O. R., Hemleben, C. & Bé, A. W. H. (in preparation).
7. Loeblich, Jr. A. R. & Tappan, H. in *Treatise on Invertebrate Paleontology* (ed. Moore, R. C.) C1-C900 (Geol. Soc. Am., New York, 1964).
8. Berger, W. H. *Mar. Geol.* **11**, 325–358 (1971).
9. Bishop, J. K. B., Edmond, J. M., Ketten, D. R., Bacon, M. P. & Silker, W. B. *Deep Sea Res.* **24**, 511–548 (1977).

Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice

SERIOUS environmental contamination is a hazard in the use of derivatives of 2,4,5-trichlorophenol (TCP) as weedkillers¹ or defoliants² and in accidents during the manufacture of TCP³ (as in Seveso, Italy in 1976^{4–6}). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) is an inevitable toxic⁷ teratogenic⁸ and weakly mutagenic^{9,10} by-product which is always present as a contaminant. When TCP derivatives, including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), were tested for carcinogenicity in (C57BL/6 × C3H/Anf)_{F1} and (C57BL/6 × AKR)_{F1} mice (experiments lasting 18 months) none increased tumour incidence¹¹, while in another study there was a significant increase in the incidence of tumours in C3Hf mice treated with 2,4,5-T¹². During a chronic toxicity study with TCDD (a potent inducer of microsomal enzymes¹³) in Sprague–Dawley rats some tumours were observed¹⁴. In another 2-yr study TCDD ingested at 0.1 µg per kg body weight per day caused an increased incidence of hepatocellular and squamous carcinomas in

Sprague–Dawley rats, but reduced the incidence of some other tumours. Smaller doses of dioxin did not affect tumour incidence¹⁵. We report here that both 2,4,5-trichlorophenoxyethanol (TCPE), a derivative of TCP, and dioxin enhance liver tumours in male mice in a dose-dependent manner. Amyloidosis is a long term complication often associated with severe skin lesions induced by TCDD.

Because TCPE is almost insoluble in water it was suspended in a salt solution containing 0.5% carboxymethylcellulose. Dioxin was dissolved in sunflower oil and determined by gas chromatography. Various doses of a combination of TCPE and dioxin, of pure dioxin and of vehicles alone were administered (Table 1). In group 1, TCPE contained 0.3% of contaminants. Ten-week-old, outbred Swiss/H/Riop mice (25–30 g) were used in groups of 100 males plus 100 females, except for groups administered pure dioxin and sunflower oil alone, each of which consisted of 45 males only. Animals were kept in plastic cages at 25 ± 2 °C. Standard diet and water were provided *ad lib*. TCPE contaminated with dioxin, pure dioxin or vehicle alone were administered by gastric tube once a week for 1 yr.

Animals were followed up for the rest of their lives. Autopsies were performed after spontaneous death or when the mice were moribund, and all organs were examined histologically. For light microscopy, sections were stained with haematoxylin and eosin. Amyloid deposits were identified with Congo red stain under polarised light¹⁶ and by electron microscopy. Pathological findings were assessed and analysed statistically. The effective number of animals was the number of survivors at the time the first tumour was observed. Significance was calculated by the χ^2 test. A difference in tumour frequency between groups was considered significant only when *P* was less than 1%. The carcinogenesis study was carried out in three phases (groups 1–3, 4–8 and 9–12) and comparisons were made with the respective control groups.

The LD₅₀ of an acute, peroral dose of TCPE containing 0.1 p.p.m. dioxin is 1,320 mg kg⁻¹. The largest dose of TCPE that induced no noticeable tissue lesions in the organs of the mice during 6 months of administration was 70 mg per kg. That was therefore taken as the maximum tolerated dose.

Table 1 Cumulative data on tumour incidence

Groups	Treatment			Sex	Effective no. of mice	No. of tumour bearing mice	Animals with tumours of				Average life span (d)
	TCPE (mg per kg)	Dioxin (µg per kg)	Vehicle* (mg per kg)				liver no. (%)	lung no.	lymphoma no.	other organs no.	
1	67.0	0.112 (1.6 ppm)	50	M	88	69	42† (48)	50	7	16	595
				F	83	61	7 (8)	52	15	25	652
2	70.0	0.007 (0.1 ppm)	50	M	98	78	57‡ (58)	48	11	16	571
				F	96	59	9 (9)	39	15	23	582
3	Control	Control	50	M	93	63	2 (26)	44	8	17	577
				F	84	57	4 (5)	41	23	18	639
4	7.0	0.07 (10 ppm)	50	M	93	79	25 (27)	54	18	22	641
				F	96	60	10 (10)	38	19	19	589
5	7.0	0.0007 (0.1 ppm)	50	M	94	77	23 (24)	50	28	17	660
				F	93	71	8 (9)	42	36	21	590
6	0.7	0.00007 (0.1 ppm)	5.0	M	97	78	24 (25)	51	20	17	643
				F	94	64	5 (5)	38	22	21	566
7	—	—	50	M	96	74	32 (33)	44	14	22	615
				F	84	55	4 (5)	38	18	17	565
8	Control	Control	50	M	96	78	32 (33)	58	22	15	651
				F	91	57	4 (4)	31	24	19	549
9	—	7.0	10	M	43	27	13 (30)	11	6	7	424
10	—	0.7	10	M	44	36	21† (48)	18	12	4	633
11	—	0.007	10	M	44	39	13 (29)	27	10	6	649
12	—	—	10	M	38	27	7 (18)	15	6	7	588

* Carboxymethylcellulose in groups 1–8, sunflower oil in groups 9–12.

† *P* < 1%.

‡ *P* < 0.1%.

Table 2 Occurrence of amyloidosis with skin lesions induced in mice by dioxin

Groups	Treatment		Effective no. of mice	Skin lesion no.	Skin lesion + amyloidosis no.
	Dioxin ($\mu\text{g per kg}$)	Vehicle (mg per kg)			
9	7.0	10.0	43	25	17
10	0.7	10.0	44	13	10
11	0.007	10.0	44	5	5
12	—	10.0	38	0	0

The cumulative data in Table 1 show that only the incidence of liver tumours of the males significantly differed between groups. Subsequent comparisons therefore relate to the males. The incidence of liver tumours in groups 1, 2 and 10 was twice that in the respective controls (groups 3 and 12). The smaller doses of TCPE and dioxin given to groups 5 and 6 (1/10 and 1/100 of maximum tolerated dose) caused less frequent tumours; there were no significant differences from groups 7 and 8. There were no significant differences in the frequency of liver tumours between groups 1 and 2 (given dioxin at 0.112 and 0.007 $\mu\text{g per kg}$) or groups 4 and 5. The latter two groups received equal amounts of TCPE but group 4 received 100 times more dioxin than did group 5. Thus, dioxin in this dose range did not influence tumour frequency.

Groups 11 and 2 received equal amounts of dioxin (0.007 $\mu\text{g per kg}$) but there was no significant difference in liver tumour incidence between group 11 and its control, group 12. Thus dioxin has no liver tumour enhancing effect at this dose. The increased incidence of liver tumours in group 2 was caused by TCPE and not by dioxin. In group 10, which received 100 times more dioxin than did group 11 (0.7 $\mu\text{g per kg}$), liver tumour incidence was twice that in the control, group 12. Thus dioxin has a liver tumour enhancing effect at a higher dose. Dioxin contamination in group 4 was less than 0.7 $\mu\text{g per kg}$ —the threshold dose of TCDD in our experiment—and its hepatocarcinogenic effect could not be observed.

Spontaneous and induced liver tumours were not histologically different. The ratio of benign hepatomas to hepatocellular carcinomas in the control groups was not affected by treatment; only the absolute number of liver tumours increased. No cirrhosis was seen concurrently with tumours.

Average life span decreased considerably in group 9 (Table 1) which received the largest dose of TCDD. TCDD caused severe chronic, ulcerous skin lesions (probably similar to chloracne in humans) followed by generalised lethal amyloidosis (Table 2), which can be regarded as a process secondary to chronic skin lesion¹⁷. This group was not evaluated in the carcinogenic bioassay for early death.

Until more is known about the people who have been exposed to them, the carcinogenicity of 2,4,5-T and structurally related chemicals in humans cannot be evaluated^{18–21}. But it is clear that not only dioxin contamination, but also exposure to TCPE during its production or use should be controlled and reduced. Amyloidosis should be considered a possible later complication of the most severe forms of human chloracne induced by TCDD.

A preliminary report of this study was presented at the International Conference on Ecological Perspectives on Carcinogens and Cancer Control in Cremona in 1977²². This work was initiated by the late Professor B. Kellner. We thank Mrs Rose Gaál Szabo for technical assistance.

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- Maier-Bode, H. *Anz. Schädlingsk.* **45**, 2–6 (1972).
- Norman, C. *Nature* **248**, 186–188 (1974).
- Economist* August 14, 73 (1976).
- Hay, A. *Nature* **262**, 636–638 (1976).
- Garattini, S. *Biomedicine* **26**, 28–29 (1977).
- Reggiani, G. *Arch. Toxicol.* **40**, 161–188 (1978).
- Schwetz, B. A. *et al. Env. Health Perspect. Exp. Issue* **5**, 87–99 (1973).
- Sparschu, G. L., Dunn, F. L. & Rowe, V. K. *Food Cosmet. Toxicol.* **9**, 405–412 (1971).
- Hussain, S., Ehrenberg, L., Lofroth, G. & Gejvall, T. *Ambio* **1**, 32–33 (1972).
- Seiler, J. T. *Experientia* **29**, 622–623 (1973).
- Innes, J. R. *et al. J. natn. Cancer Inst.* **42**, 1101–1114 (1969).
- Muranyi-Kovacs, I., Rudali, G. & Imbert, J. *Br. J. Cancer* **33**, 626–633 (1976).
- Niwa, A., Kumaki, K. & Nebert, D. W. *Molec. Pharmacol.* **11**, 399–408 (1975).
- Van Miller, J. P., LaLich, J. J. & Allen, J. R. *Chemosphere* **9**, 537–544 (1977).
- Kociba, R. J. *et al. Toxicol. Appl. Pharmac.* **45**, 298 (1978).
- Romhányi, G. *Zbl. allg. Path. path. Anat.* **80**, 411 (1943).
- Glenner, G. G. & Page, D. L. in *International Review of Experimental Pathology* (eds Epstein, M. A. & Richter, G. W.) 2–80 (Academic, New York, 1976).
- IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals **15**, 41–103, 111–139, 273–301 (IARC, Lyon, 1977).
- Hay, A. *Nature* **269**, 468–470 (1977).
- Hay, A. *Nature* **269**, 749–750 (1977).
- Hay, A. *Nature* **271**, 597–598 (1978).
- Tóth, K., Sugár, J., Somfai-Relle, S. & Bence, J. *Prog. biochem. Pharmac.* **14**, 82–93 (Karger, Basel, 1978).

Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial

MOST macropodid marsupials conceive at postpartum oestrus and the 100-cell embryo enters into and remains in embryonic diapause while there is a suckling young in the pouch¹. Removal of the pouch young results in reactivation of the quiescent corpus luteum and of the diapausing blastocyst. Prolactin inhibits corpus luteum development tonically during lactational quiescence in the tammar wallaby, *Macropus eugenii*². However, it is not known whether the hormonal milieu associated with lactation, or the neural stimulus of suckling itself is the primary signal to the hypothalamus which results in the maintenance of embryonic diapause in *M. eugenii*. I therefore examined the role of the neural pathway from the mammary gland in tammar wallabies carrying diapausing embryos. I report here that after denervation of the suckled mammary gland, the quiescent corpus luteum and the diapausing blastocyst resumed development, whereas none of a sham-operated group initiated blastocyst development. The pouch young grew at a normal rate and continued to obtain milk from denervated glands, despite the presence of a developing embryo in the uterus.

Only one of the four available teats is suckled by a particular pouch young. Each teat has a separate mammary gland, but the anterior and posterior teats on each side are less than 1 cm apart³. In experimental animals, the suckled gland and its ipsilateral partner were chosen for denervation, whereas, because of the diffuse nature of the innervation, in control animals the contralateral pair were denervated.

Denervation was effected by cutting around the chosen pair of nipples, and clearing the gland from the connective tissue until the ilio marsupialis muscle, through which the mammary nerves pass, was clearly visible. The minor blood vessels and ducts leading to the mammary lymph glands were ligated, the muscle layer cut through, and the remaining fascia and connective tissue cleared from the entire gland until it was connected only by the intact major artery and vein and there was no sign of any remaining nerves. After repair of the wound the pouch young, aged either 35 or 60 d, was replaced on the nipple.

Pouches were checked daily for the first 7–10 d. Subsequently, the females were replaced in their outdoor enclosures until laparotomy on either day 22 or 23 post-denervation.

After laparotomy, all control and pregnant experimental animals were killed. The corpora lutea were weighed directly, and embryos measured and aged, as previously described^{4,5}.

Table 1 Influence of denervation of the mammary gland on embryonic diapause; status of embryos 22 d after denervation of the suckled (experimental) or non-suckled (control) mammary gland

No. of animals	Pouch young status	Blastocyst or vesicle recovered	Day 22/23 embryos	Developed§ CL or follicle but no embryo	No blastocyst,§ unfertilised egg, CL or follicle	Total activated after operation
Experimental						
9	5 remained on teat 4 lost 5–14 d	0 0	3* 1*	1 2	1 1	7/9
Control						
4	3 remained on teat 1 lost 9 d	2† 1‡	0 0	0 0	1 0	0/4

Anaesthesia was induced by 2.0 ml sodium pentobarbitone (60 mg ml⁻¹) in 0.9% NaCl, maintained by halothane in oxygen. CL, corpus luteum.

* Embryo sizes (crown–rump length), 10 mm, 14.8 mm, 13.3 mm, 11.1 mm. † Blastocyst sizes (diameter), 0.25 mm, 0.26 mm. ‡ Vesicle size (diameter), 0.6 mm.

§ Three of the five denervated animals which were not pregnant underwent an infertile cycle, and had a developing follicle and enlarged corpus luteum at laparotomy. The remaining two denervated animals and one of the four controls had no apparent postpartum ovulation, as there was no corpus luteum in the ovary, and no egg or blastocyst in the uterus.

Mammary glands were fixed in buffered formalin for histological examination. Non-pregnant experimental animals were examined by laparotomy for post-luteal follicular development or corpora lutea and allowed to recover.

After denervation of the suckled mammary gland, the quiescent corpus luteum resumed development, as indicated by increased size and weight, and post-luteal follicular growth was observed in seven of nine experimental animals (Table 1). In four of nine animals embryos developed despite the presence of a suckling pouch young. Twenty-one days after denervation the embryos of these four fitted within the size range of normal 22-d embryos⁵. The five pouch young which remained on the denervated glands grew normally, and when measured and aged on a growth curve⁶ all had maintained a normal growth rate. Pouch young were lost in four cases. Histological examination of the glands confirmed that the nerve supply remained severed. In the

control group, none of the four animals initiated development, although one which lost its pouch young between 9 and 13 d after the operation carried a 10-d vesicle at laparotomy on day 22 (Table 1). Thus, surgery itself did not cause resumption of development.

These results show that the neural stimulus derived from suckling is essential to maintain the quiescent state of the diapausing blastocyst, but that an intact mammary neural connection is not essential for maintenance of this stage of lactation in this species. Milk secretion after denervation continued apparently unimpaired in five of the nine experimental animals. Histologically, the glands seemed normal when compared with glands obtained from animals carrying pouch young of the same age (60–84 d). However, it is not possible to say whether the remaining four were lost (at days 5, 11, 12 and 14 post-denervation) due to a failure of lactation (such as the absence of oxytocin and the milk ejection reflex), or to post-operative behavioural abnormalities. Milk was observed slowly leaking from the denervated teat before replacement of the pouch young, and in animals which lost their pouch young the gland became swollen. In other species, the myo-epithelium can be induced to contract by rapid mechanical stretching⁹; pouch young could certainly pull the denervated teat during their movements in the pouch and this may counteract the lack of oxytocin release. Oxytocin in *M. eugenii* thus may be a facultative but not obligatory mechanism for effective milk discharge, as Cross⁹ suggests for the goat.

The ability of denervation to allow the corpus luteum to resume growth, and lactation to continue, contrasts with the results of hypophysectomy in this species, in which the corpus luteum also resumes development but lactation and follicular growth fail¹⁰. However, lactation continues after removal of the corpus luteum although follicular development begins and animals return to oestrus 11 d later². It must be concluded that suckling, mediated through the neural pathway and prolactin, inhibits the corpus luteum, and that it is corpus luteum secretions (such as progesterone or oestradiol-17β) which inhibit subsequent follicular development in the intact animal.

These findings provide evidence for the role of the neural pathway from the mammary gland as a primary signal to the hypothalamus in maintaining embryonic diapause during lactation in a marsupial species. Figure 1 shows how this finding is consistent with present evidence for the control of reproductive activity in this species during lactational quiescence.

John Leonard helped develop the denervation technique, and Professor R. H. Dunlop made useful suggestions. I thank Drs M. Peaker, R. V. Short and R. B. Heap for discussions, and Stuart Green for assistance. Financial support was provided by the NIH (NICHD, HD 09387) and the Australian Research Grants Committee (D1-95/18759).

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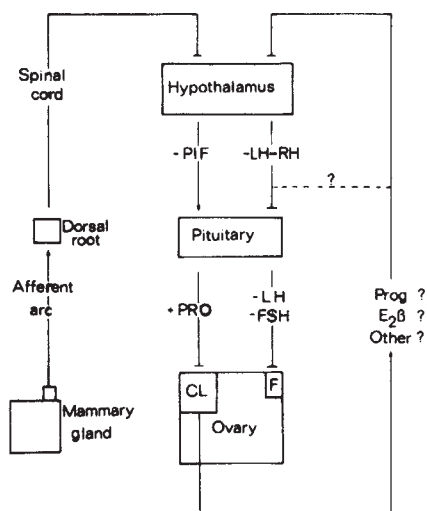


Fig. 1 An hypothetical scheme showing pathways consistent with present evidence for the control of lactational quiescence in the tammar wallaby. Removal of the pouch young and thereby the suckling stimulus leads to resumption of corpus luteum growth and of embryonic development, with oestrus and ovulation occurring at the end of the 27-d pregnancy¹. Removal of the pituitary leads to resumption of corpus luteum and blastocyst growth but ovulation and follicular development fail¹⁰. Hypophysectomy followed by prolactin injection prevents resumption of corpus luteum development², and bromocriptine administration in intact animals results in reactivation of the corpus luteum¹¹. Removal of the corpus luteum leads to oestrus and ovulation 12 d later², presumably due to the removal of small amounts of steroids derived from the quiescent corpus luteum that may inhibit pituitary gonadotrophin secretion. It is not clear, however, whether such corpus luteum inhibition acts directly on the pituitary, or through the hypothalamus, as suggested elsewhere^{12,13}. CL, corpus luteum; E₂β, oestradiol-17β; F, follicle; FSH, follicle-stimulating hormone; LH, luteinising hormone; LH-RH, luteinising hormone releasing hormone; PIF, prolactin inhibiting factor; PRO, prolactin; Prog, progesterone; †, stimulation; ‡, inhibition; +, increased levels; -, decreased levels.

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1. Tyndale-Biscoe, C. H., Hearn, J. P. & Renfree, M. B. *J. Endocr.* **63**, 589–614 (1974).
2. Tyndale-Biscoe, C. H. & Hawkins, J. in *Reproduction and Evolution* (eds Calaby, J. H. & Tyndale-Biscoe, C. H.) 245–252 (Australian Academy of Science, 1977).
3. Griffiths, M., McIntosh, D. L. & Leckie, R. M. C. *J. Zool. Res.* **166**, 265–275 (1972).
4. Renfree, M. B., Green, S. W. & Young, I. R. *J. Reprod. Fert.* (submitted).
5. Renfree, M. B. & Tyndale-Biscoe, C. H. *Dev. Biol.* **32**, 28–40 (1973).
6. Murphy, C. R. & Smith, J. R. *Trans. R. Soc. S. Aust.* **94**, 15–20 (1970).
7. Denamur, R. & Martinet, J. *C. r. heb. Séanc. Soc. Biol.* **148**, 833–836 (1954).
8. Linzell, J. L. *Q. Jl exp. Physiol.* **48**, 34–60 (1963).
9. Cross, B. A. *Symp. Zool. Soc. Lond.* **41**, 193–210 (1977).
10. Hearn, J. P. *Nature* **241**, 207–208 (1973).
11. Tyndale-Biscoe, C. H. *Ciba Fdn Symp.* (in the press).
12. Short, R. V. *Ciba Fdn Symp.* **45**, 73–86 (1973).
13. Hearn, J. P., Short, R. V. & Baird, D. T. in *Reproduction and Evolution* (eds Calaby, J. H. & Tyndale-Biscoe, C. H.) 255–263 (Australian Academy of Science, 1977).

Abelson virus-transformed haematopoietic cell lines with pre-B-cell characteristics

THE Abelson murine leukaemia virus (A-MuLV) complex, which consists of a replication-defective focus-forming virus and its helper Moloney leukaemia virus, causes a thymus-independent lymphosarcoma in BALB/c mice¹. Infection *in vivo* leads to the production of tumours which are usually classified as 'null' lymphoid cells², although immunoglobulin (Ig)-synthesising B-cell lines have been reported^{3–5} and macrophage lines may occasionally be produced⁶. In some cases, particularly with pretreatment of the mice with the mineral oil pristane, there is a high incidence of Ig-producing plasmacytomas⁷. Additional data on Ly phenotypes have suggested that most A-MuLV tumours, including those with no demonstrable Ig synthesis, may be B-cell lineage derivatives⁸. Here, we provide evidence for A-MuLV transformation of B-cell precursors resulting in two cell lines which retain the capacity for inducible IgM synthesis. Line ABC-1 was derived from BALB/c mouse bone marrow cells transformed *in vitro* by the Abelson virus⁹ and line ATV-3 from a tumour in a BALB/c mouse inoculated with A-MuLV.

The ABC-1 cells are large, vacuolated, undifferentiated blast cells. Immunofluorescence and cytotoxicity tests indicated that no cells had cell surface immunoglobulin (SmIg) or expressed detectable levels of Thy-1.2, suggesting that there were no mature B or T lymphocytes present. Additional characterisation of this cell line (Table 1) revealed that no cells expressed Ia or TL 1,2,3,4 antigens or peanut agglutinin binding sites (Gal(β1-3)-GalNAc), the latter being a marker for thymocytes and T-cell precursors¹⁰. All cells contained cell surface exposed G_{M1} gangliosides as indicated by binding of ¹²⁵I-cholera toxin or fluoresceinated cholera toxin. The cells did not react with a rabbit antiserum specific for antigens on a gp100 molecule associated with a human non-T, non-B or 'null' lymphoblastic leukaemia^{11,12}. Preliminary experiments suggest that the ABC-1 cells, in common with other A-MuLV-transformed lines⁸, have low but detectable levels of terminal deoxynucleotidyl transferase (M.B., unpublished observations).

The use of affinity-purified antibodies to mouse IgM conjugated with fluorescein indicated an absence of cells with detectable cell surface fluorescence, although 1–4% showed cytoplasmic staining. No cytoplasmic fluorescence was seen in controls of AKR leukaemic T lymphocytes and normal thymocytes. In normal spleen, plasma cells stained intensely.

The presence of cytoplasmic IgM in a minor proportion of cells and the absence of staining for surface membrane Ig indicate the presence of cells with the characteristics of the earliest detectable cells in the B-cell lineage, that is, 'pre-B' cells^{13,14}.

We next attempted to induce lymphoid differentiation in ABC-1 cells which had been in continuous culture for only 8 months. Dimethyl sulphoxide (DMSO), 1%, failed to induce any increase in the percentage of cells expressing cytoplasmic

Table 1 Surface membrane phenotype of an *in vitro* Abelson virus-transformed line ABC-1

Marker	% Positive cells		
	A-MuLV line: ABC-1	Controls (BALB/c) Spleen	Thymus
SmIg	<0.01	40	2
Thy-1.2	<1	19	83–95
Ia	<1	30–45	3
TL 1,2,3	<5	<5	11
TL 4	<5	<5	46
Peanut agglutinin	1	3	>95
Cholera toxin receptors	100	100	>95
Fc receptors	25	34	5
Human gp100 cALL antigen	<0.1	ND	<0.1

Surface membrane immunoglobulin (SmIg) was detected by direct immunofluorescence using several anti-Ig antisera, including rabbit anti-mouse Ig (non-class specific) and a rabbit antiserum specific for IgM. Thy-1.2 was determined by indirect immunofluorescence using an AKR anti-CBA thymocyte antiserum (Searle) followed by a fluorescein-labelled rabbit anti-mouse Ig and a two-stage ⁵¹Cr-release cytotoxicity test using the same anti-Thy-1.2 antiserum plus rabbit complement (1:15). Values given refer to results by ⁵¹Cr release and represent the percentage specific lysis. Ia expression was tested by indirect immunofluorescence techniques and a two-stage cytotoxicity test (using trypan blue exclusion). Anti-Ia was a (B10.A×A) anti-B10.D2 antiserum (anti-Ia^d). Values for controls are derived from indirect immunofluorescence experiments in which it was necessary first to remove surface membrane Ig of spleen cells by 'capping'. TL antigens were detected by complement-mediated cytotoxicity. Anti-TL antisera were produced by immunisation of appropriate congenic strains of mice. Anti-TL-4 was raised by immunising (A×C57BL/6-Tla^a) mice with a C57BL thymic lymphoma line (ERLD). Anti-TL 1,2,3 was raised by immunising (A-Tla^b×C57BL/6) with a thymic lymphoma line (ASL1) derived from an A strain mouse. cALL, common acute lymphoblastic leukaemia antigen. ND, not determined.

Table 2 Stimulation of cytoplasmic IgM production in an *in vitro* transformed line ABC-1

Treatment	% cells staining for cytoplasmic IgM on			
	Day 0	Day 1	Day 4	Day 7
None	1–4	1–4	1–4	1–4
<i>Escherichia coli</i> LPS				
5 μg ml ⁻¹	—	20	23	25
20 μg ml ⁻¹	—	25	39	25
Dimethyl sulphoxide, 1%	—	ND	23	4
Butyric acid, 1 mM	—	1	18	4
Pokeweed mitogen conditioned medium	—	7	34	ND

Concentrations of each inducer shown are those which, out of the range of concentrations used, were found to induce maximal stimulation of cytoplasmic IgM-positive cells. Pokeweed mitogen conditioned medium refers to supernatant from syngeneic spleen cell cultures (5×10⁶ fresh cells ml⁻¹) incubated for 72 h with pokeweed lectin(s) (15 μg ml⁻¹). The cells were removed by centrifugation (1,300 g) and the conditioned medium used at one part conditioned medium to two parts fresh medium. Abelson virus-transformed cell cultures were set up at 10⁶ cells per ml on day 0 and assayed on days 1, 4 and 7 for the presence of surface membrane Ig and Thy-1.2 by fluorescence techniques (see legend Table 1). Thy-1.2 was also assayed on five occasions by a ⁵¹Cr-release cytotoxicity test. Smears were also made and stained with anti-IgM antibodies (see legend to Fig. 1). Rabbit anti-mouse IgM (μ-chain specific) antiserum was raised by immunisation with purified MOPC 104E myeloma protein (Ig). The rabbit antibodies were isolated on a MOPC 104E-4B Sepharose immunoabsorbent column, passed over mouse myeloma IgG-4B Sepharose to remove anti-γ chain cross-reactivity and conjugated to fluorescein or rhodamine isothiocyanate.

IgM or *de novo* expression of cell surface Ig. Lipopolysaccharide (LPS), at doses of $5\text{--}20\ \mu\text{g ml}^{-1}$, rapidly induced an increased expression of cytoplasmic IgM, maximally 9–10% 2 d after addition (data not shown).

Cells which had been in continuous culture for longer (16 months) showed a more pronounced stimulation of cytoplasmic IgM-positive cells (20–40%). Several compounds were tested for their ability to elicit differentiation; these were added over a range of doses to fresh cultures (Table 2). Cells were assayed on days 1, 4 and 7 for the presence of Thy-1.2, SmIg and cytoplasmic IgM. DMSO, LPS, butyric acid and conditioned medium from BALB/c spleen cell cultures stimulated with pokeweed lectin(s) (PWM), all increased the percentage of cytoplasmic IgM-positive cells. No cell surface Ig-positive cells were detectable after these treatments. Of the compounds tested, only LPS induced an increase in cells with cytoplasmic IgM production within 1 d, but there was no significant difference in proliferation over this period ($8.7 \times 10^5\ \text{ml}^{-1}$ and $9.3 \pm 0.3 \times 10^5\ \text{ml}^{-1}$ for control and LPS-stimulated cells, respectively). Stimulation by butyric acid and PWM conditioned medium was not evident at day 1 but was seen at day 4.

In contrast to the responses elicited by the above compounds, dextran sulphate ($2\text{--}50\ \mu\text{g ml}^{-1}$), concanavalin A ($5\text{--}10\ \mu\text{g ml}^{-1}$), PWM ($5\ \mu\text{g ml}^{-1}$), dibutyryl cyclic AMP

($1.25\text{--}5 \times 10^{-4}\ \text{M}$), cholera toxin ($0.01\text{--}1.0\ \mu\text{g ml}^{-1}$), phytohaemagglutinin ($2.5\text{--}20\ \mu\text{g ml}^{-1}$) and purified protein derivative of tuberculin ($5\text{--}50\ \mu\text{g ml}^{-1}$) all failed to stimulate an increase in cells with cytoplasmic IgM, Thy-1.2 or SmIg.

Several cell lines were also isolated from tumours in BALB/c mice which had been injected intraperitoneally at 5–7 d of age with Abelson virus. All these lines were negative for Thy-1.2 and SmIg. We attempted to induce lymphoid differentiation in two lines in which there were no cells showing detectable cytoplasmic fluorescence with anti-IgM; DMSO, dextran sulphate, LPS, thymopoietin, iododeoxyuridine, and interferon ($475\ \text{units ml}^{-1}$) with or without 1% DMSO or dextran sulphate, did not induce expression of Thy-1.2, cell surface Ig or cytoplasmic IgM.

A third line (ATV-3) had 5–22% (mean 15%) cells with cytoplasmic IgM, none of which had detectable cell surface immunoglobulin. After 48–72 h culture with $10\ \mu\text{g ml}^{-1}$ LPS the proportion of cells with cytoplasmic IgM had increased to 35% and 70% in two experiments, with no concomitant appearance of cell surface Ig.

Synthesis of IgM by both ABC-1 and ATV-3 lines was confirmed by SDS-polyacrylamide gel electrophoresis and fluorography (Fig. 1). Both lines apparently synthesised μ heavy chain and light chain.

The synthesis of IgM by these lines and the induction of increased numbers of detectable IgM-containing cells imply that A-MuLV had transformed immature B-lymphoid lineage committed precursors. A minority of cells in both lines seems to have a maturation arrest at the level of 'pre-B' cells whereas a substantial proportion are presumably less mature but can be induced to express pre-B features. No *bona fide* 'mature' B cells (cell surface Ig positive) have been inducible in these lines. It is interesting that LPS and PWM-activated cell supernatants induce pre-B-cell characteristics in ABC-1 cells, whereas concanavalin A, phytohaemagglutinin or pokeweed lectin itself do not, as this parallels the differential mitogenic effects of these same compounds on purified murine T and B cells *in vitro*¹⁵. Previous work has indicated that polyclonal B-cell activators may selectively depress DNA synthesis of A-MuLV-transformed cells¹⁶. LPS has also been reported to stimulate a chemically induced pre-B-cell line to express cell surface Ig¹⁷. These data and analyses of Ly antigen phenotypes⁸ support the view that A-MuLV-transformed cells are predominantly B-lineage committed cells.

Baltimore *et al.* have recently provided further evidence for B-cell differentiation in several A-MuLV-transformed lines^{18,19}, some of which are similar to the two reported here in synthesising μ chains with no cell surface representation¹⁸. These lines apparently synthesised light chains only following stimulation with LPS^{18,19}.

Abelson virus therefore seems to transform mainly B-lineage lymphoid cells. In the two lines reported here in detail, transformation *in vitro* (ABC-1 line) or *in vivo* (ATV-3 line) resulted in the production of lines with features indicative of early maturation compartments in the B-lymphocyte lineage. The availability of such cells with at least a partial competence for reversal of maturation arrest should be useful for studying early molecular events in the control of immunoglobulin gene diversification and expression.

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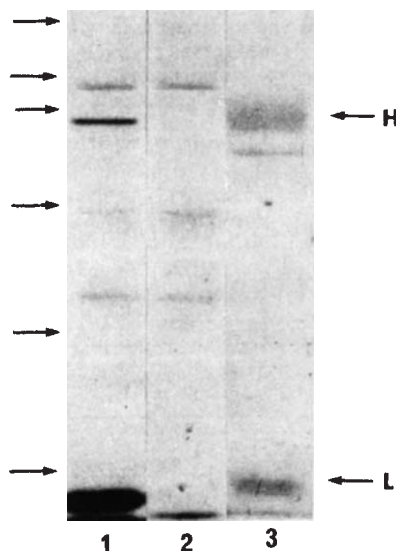


Fig. 1 Fluorography of SDS-polyacrylamide gel electrophoresis showing μ heavy and light chain synthesis by Abelson virus-transformed cells (ATV-3). Slot 1, immune precipitate with rabbit anti-mouse μ chain antibodies; slot 2, immune precipitate with normal rabbit serum; slot 3, Coomassie blue-stained tract containing MOPC-104E IgM. Positions of heavy (H) and light (L) chains from MOPC-104E indicated. Molecular weight markers (arrowed) are (from top to bottom): β -galactosidase (135,000); phosphorylase a (94,000); lactoperoxidase (80,000); catalase (60,000); alcohol dehydrogenase subunit (yeast, 37,500) and carbonic anhydrase (29,000). 1×10^8 cells were incubated at 37°C for 16 h with $250\ \mu\text{Ci } ^{35}\text{S}$ -methionine in methionine-free medium. The labelled cells were washed three times in methionine-free medium and extracted in 3 ml 0.01 M Tris, pH 8, 150 mM NaCl and 0.5% NP40 for 15 min at 4°C . The cell extract was centrifuged at $100,000g$ for 1 h at 4°C ; $250\ \mu\text{l}$ were incubated overnight with $20\ \mu\text{l}$ of rabbit anti-mouse μ chain or normal rabbit serum and the immune complexes isolated by *Staphylococcus aureus* precipitation. The bacteria were pelleted, washed three times in the above buffer with 0.25% gelatin, and bound complexes were eluted by boiling in $70\ \mu\text{l}$ of 10% glycerol, 2% SDS, 0.05 M Tris, pH 6.8, plus $10\ \mu\text{l}$ 1 M dithiothreitol in 0.1% bromophenol blue indicator. The eluted complexes as well as the reduced MOPC-104E and marker proteins were subjected to SDS-PAGE.

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- Abelson, H. T. & Rabstein, L. S. *Cancer Res.* **30**, 2213–2222 (1970).
- Pratt, D. M. *et al.* *Cell* **12**, 683–690 (1977).
- Rosenberg, N., Baltimore, D. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1932–1936 (1975).
- Premkumar, E., Potter, M., Singer, P. A. & Sklar, M. D. *Cell* **6**, 149–159 (1975).
- Weimann, B. J. *Cold Spring Harb. Symp. quant. Biol.* **41**, 163–164 (1977).
- Raschke, W. C., Baird, S., Ralph, P. & Nakoinz, I. *Cell* **15**, 261–267 (1978).
- Potter, M., Sklar, M. D. & Rowe, W. P. *Science* **182**, 592–594 (1973).
- Silverstone, A. E. *et al.* in *Cold Spring Harb. Conf. Cell Proliferation* Vol. 5 (eds Clarkson, B., Till, J. E. & Marks, P. A.) 432–452 (Cold Spring Harbor Press, New York, 1978).
- Teich, N., Boss, M. & Dexter, T. M. in *Modern Trends in Human Leukaemia* Vol. 3 (eds Neth, R., Hofschneider, P.-H. & Mannweiler, K.) (Springer, Berlin, in the press).
- London, J. & Roelants, G. E. in *25th Colloq. Protides of the Biological Fluids* (Pergamon Press, 1977).
- Sutherland, D. R., Smart, J., Niaudet, P. & Greaves, M. F. *Leukaemia Res.* **2**, 115–126 (1978).
- Brown, G., Capellaro, D. & Greaves, M. F. *J. natn. Cancer Inst.* **55**, 1281–1289 (1975).
- Raff, M. C., Megson, M., Owen, J. J. T. & Cooper, M. D. *Nature* **259**, 224–226 (1976).
- Melchers, F., Andersson, J. & Phillips, R. A. *Cold Spring Harb. Symp. quant. Biol.* **41**, 147–150 (1977).
- Janossy, G. & Greaves, M. F. *Transplant Rev.* **24**, 177–236 (1975).
- Ralph, P., Nakoinz, I. & Raschke, W. C. *Biochem. biophys. Res. Commun.* **61**, 1268–1275 (1974).
- Paige, C. J., Kincade, P. W. & Ralph, R. J. *Immun.* **121**, 641–647 (1978).
- Siden, E. J., Baltimore, D., Clark, D. & Rosenberg, N. *Cell* (in the press).
- Rosenberg, N., Siden, E. & Baltimore, D. in *B Lymphocytes and the Immune Response* (eds Cooper, M., Mosier, D., Scher, I. & Vitetta, E.) (Elsevier, Amsterdam, in the press).

Retinoic acid inhibition of Epstein-Barr virus induction

TUMOUR promoters of the diterpene type efficiently induce Epstein-Barr virus (EBV) and additional persisting herpes viruses in genome-harboring lymphatic cells^{1,2}. The tumour-promoting activity of these substances to some extent parallels their efficiency in virus induction²: the most active tumour promoters induce EBV even at concentrations of 10^{-10} – 10^{-9} M, whereas 100–500-fold higher concentrations are required for induction by weak promoters. Treatment of cells with tumour promoters results in a fast and reproducible increase in the activity of ornithine decarboxylase (ODC)^{3,4} up to 250-fold levels of normal activity. Recent reports^{5,6} described the inhibition of ODC induction by pretreating the cells with retinoic acid. Moreover, retinoic acid has been reported to act as an inhibitor in experimental 2-stage carcinogenesis models⁷. We have therefore investigated the possible interaction of retinoic acid with virus induction by tumour promoters. The effect of retinoic acid was also studied on the induction of early antigens (EA) of EBV by the pyrimidine analogue, iododeoxyuridine (IUdR)^{8,9}, by treatment of IgM-producing cells with anti-IgM antibodies¹⁰ and by superinfection with EBV from P3HR-1 cells^{11,12}. The data indicate that retinoic acid specifically interferes with induction by non-viral reagents without affecting EBV antigen synthesis after superinfection.

cis-Retinoic acid (Nordmark) was dissolved in ethanol diluted in tissue culture medium and applied to Raji cells in concentrations ranging between 10^{-8} and 5×10^{-5} M. No cellular growth inhibition was noted up to 10^{-5} M; at 5×10^{-5} M there was a slight decrease in growth over a period of 5 d.

Concomitant treatment of these cells with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and retinoic acid resulted in effective inhibition of EA induction by TPA (Fig. 1). At 10^{-5} M, more than 90% of EA induction by TPA was suppressed and some inhibition was still obvious at 10^{-8} M. Kensler *et al.*⁶ reported that pretreatment of the cells with retinoic acid 1 h before TPA addition was most effective in inhibiting ODC induction. We therefore carried out analogous experiments, but found no difference in the inhibition of the EA response, regardless of whether the cells were pretreated or simultaneously treated with retinoic acid. Pretreatment of the cells with retinoic acid (10^{-6} M) for 12 h followed by subsequent washing and addition of TPA still resulted in more than 90% inhibition of EA induction. Additional experiments with retinoic acid, using a different tumour promoter (Pimelea factor P2), yielded analogous results to TPA treatment.

We then investigated the specificity of the inhibitory effect of retinoic acid on EBV antigen induction by tumour promoters. Raji cells induced by IUdR or anti-IgM-antiserum to EA synthesis were treated simultaneously with varying concentrations of retinoic acid (Figs 2, 3). Although IUdR induction of EA seems to be less strongly inhibited than TPA induction, some inhibition is obvious over a relatively wide range of retinoic acid concentration. EA induction by anti-IgM is clearly inhibited by retinoic acid.

Further experiments were designed to test the influence of retinoic acid on EA induction by superinfecting Raji cells with EBV derived from P3HR-1 cells^{11,12}. Retinoic acid did not affect the percentage of EA-positive cells, even when applied at 10^{-5} M. Similarly, the spontaneous induction rate of P3HR-1 and B95-8 cells, which is approximately 6 and 4%, respectively, was not significantly altered by applying retinoic acid at 10^{-7} – 10^{-5} M for 3 and 5 d.

These data show that retinoic acid inhibits virus induction by chemical inducers and anti-IgM antiserum without affecting antigen synthesis after superinfection or spontaneous induction. This suggests that the different inducing reagents activate the persisting genomes indirectly by a common mediator which seems to be inhibited by retinoic acid. EA induction by superinfection or spontaneous induction of viral antigens in tissue culture cells apparently follow different pathways. Such observations are consistent with the recent demonstration of a correlation between inducibility by promoters, IUdR and anti-IgM antiserum and EBV genome equivalents per cell (K.B. *et al.* in preparation), whereas the response to superinfection seems to be independent of genome numbers in EBV genome-carrying cells.

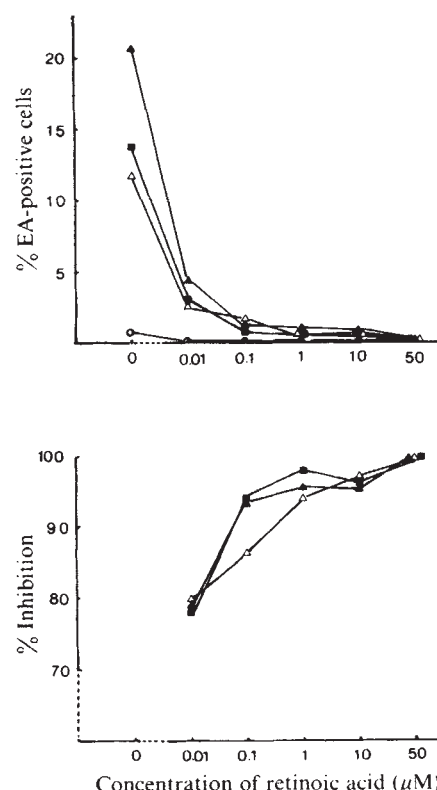


Fig. 1 Effect of retinoic acid on induction by TPA in Raji cells (clone no. 7, K.B. *et al.*, in preparation). The cells (5×10^5 per ml) were incubated in 10 ml medium (RPMI 1640 containing 10% fetal calf serum) in the presence of 20 ng ml^{-1} TPA and various concentrations of retinoic acid. The % of EA-positive cells was determined at indicated times by indirect immunofluorescence¹¹. a, The % of EA-positive cells; b, the % of inhibition in the presence of retinoic acid compared with the control cultures (without retinoic acid). ○, Day 1; ■, day 3; ▲, day 5; △, day 7.

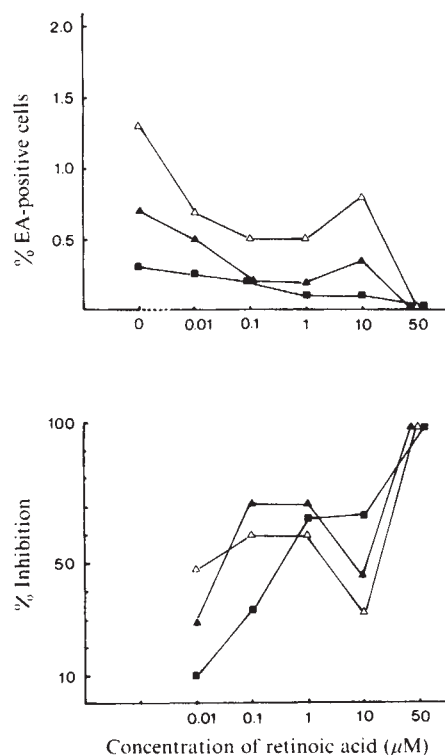


Fig. 2 Effect of retinoic acid on EA induction by IUdR in Raji cells (clone no. 7): 10 ml of the cells (5×10^5 per ml) were incubated in the presence of $100 \mu\text{g ml}^{-1}$ IUdR and various concentrations of retinoic acid. ■, Day 3; ▲, day 5; ◇, day 7.

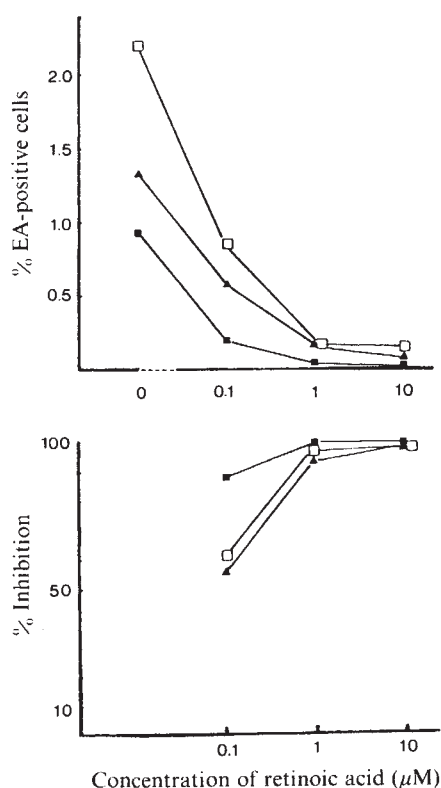


Fig. 3 Effect of retinoic acid on EA induction by anti-IgM antiserum. Raji cells were incubated with 1% of rabbit anti-human IgM antiserum (Behring-Werke) and retinoic acid in conditions outlined in Figs 1 and 2. ■, Day 1; ▲, day 2; ◇, day 4.

It is uncertain whether the inhibition of ODC activity by retinoic acid has any direct relationship to inhibition of virus induction by retinoic acid. Preliminary data indicate that neither putrescine, which should interact with ODC by feedback inhibition^{13,14}, nor colchicine, which is also reported to inhibit TPA-induced ODC activity¹⁵, reduce the number of TPA-induced Raji cells (H.z.H., unpublished data). This seems to dissociate virus induction from ODC stimulation and stresses the specificity of the virus induction process by diterpene esters.

If herpes virus persistence in general is affected by the application of retinoic acid, this could have important implications for the prevention of recurrent herpetic infections.

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- zur Hausen, H., O'Neill, F., Freese, U.-K. & Hecker, E. *Nature* **272**, 373-375 (1978).
- zur Hausen, H., Bornkamm, G. W., Schmidt, R. & Hecker, E. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- O'Brien, T. G., Simsiman, R. C. & Boutwell, R. K. *Cancer Res.* **35**, 2426-2433 (1975).
- O'Brien, T. G. *Cancer Res.* **36**, 2644-2653 (1976).
- Verma, A. K. & Boutwell, R. K. *Cancer Res.* **37**, 2196-2201 (1977).
- Kensler, T. W., Verma, A. K., Boutwell, R. K. & Mueller, G. C. *Cancer Res.* **38**, 2896-2899 (1978).
- Shamberger, R. J. *J. natn. Cancer Inst.* **47**, 667-673 (1971).
- Hampar, B., Derge, J. G., Martos, L. M. & Walker, G. L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 78-82 (1972).
- Gerber, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 83-85 (1972).
- Tovey, M. G., Lenoir, G. & Begon-Lours, *Nature* **276**, 270-272 (1978).
- Henle, W. *et al. Science* **169**, 188-190 (1970).
- Henle, G., Henle, W. & Klein, G. *Int. J. Cancer* **8**, 272-282 (1971).
- Tabor, H. & Tabor, C. W. *J. biol. Chem.* **244**, 2286-2292 (1969).
- Tabor, C. W. & Tabor, H. A. *Rev. Biochem.* **45**, 282-206 (1976).
- O'Brien, T. G., Simsiman, R. C. & Boutwell, R. K. *Cancer Res.* **36**, 3766-3770 (1976).

Teratocarcinomas rarely develop from embryos transplanted into athymic mice

SEVEN-DAY-OLD mouse embryos transplanted to extra-uterine sites give rise to tumours that have been classified as either benign teratomas or malignant teratocarcinomas¹. The ratio of teratomas to teratocarcinomas varies from one mouse strain to another, suggesting the existence of teratocarcinoma-permissive and nonpermissive strains². Control of teratocarcinogenesis in this tumour system resides in the adult recipient of the embryonic graft and might involve the immune response. We report here that we have tested the hypothesis that the absence of cell-mediated immunity enhances the growth of transplanted embryos and facilitates the development of teratocarcinoma. Contrary to our expectations, our data indicate that embryos grafted into recipients with partial or total deficiency of cell-mediated immunity grow poorly and develop almost exclusively into benign teratomas.

We compared the outgrowth of 7-d-old embryos derived from teratocarcinoma-permissive (C3H/HeJ) and teratocarcinoma-nonpermissive (C57BL/6J) mouse strains. These embryos were transplanted under the kidney capsule of normal syngeneic mice, thymectomised, irradiated and bone marrow-reconstituted (TIR) mice, and athymic (*nu/nu*) mice. Results are summarised in Table 1.

Tumours obtained in the syngeneic recipients were as previously described². In C3H/HeJ mice, two out of three of the

Table 1 Weight of tumours and the ratio of teratomas to teratocarcinomas obtained from 7-d-old embryos transplanted under the kidney capsule of adult recipients

Embryo	Recipient	No. of grafts	Weight of tumours (mg $\pm$ s.e.m.)	Teratoma/teratocarcinoma
C3H	C3H, untreated	94	3,569 $\pm$ 330 (a)	33/61 (e)
	C3H, TIR	21	557 $\pm$ 117 (b)	17/4 (f)
	Nude, nu/nu	14	814 $\pm$ 240 (b)	10/4 (g)
C57BL	C57BL, untreated	71	2,178 $\pm$ 279 (c)	64/5 (h)
	Nude, nu/nu	21	538 $\pm$ 97 (d)	20/1 (j)

An embryonic portion of 7-d-old embryos was isolated and transplanted under the kidney capsule of adult recipients which were: untreated, 2-month-old C3H/HeJ and C57BL/6J; athymic; thymectomised, irradiated and bone marrow-reconstituted (TIR) mice. Athymic (nu/nu) mice were on BALB/c background. TIR animals were thymectomised as weanlings, irradiated 2 weeks after thymectomy with 850 rad and reconstituted with 2×10^7 syngeneic bone marrow cells. Animals that survived and recovered completely were used as recipients 4 weeks after reconstitution. Recipient animals were killed 2 months after grafting. Diagnosis of teratoma and teratocarcinoma was made histologically. *P* value, as calculated by Student's *t* test, was <0.005 for *a* relative to *b*, *c* to *d*, and *a* to *c*. *P* values, as calculated by χ^2 test, were as follows: <0.005 (*e* relative to *f*), <0.025 (*e* to *g*), >0.75 (*f* to *g*), <0.005 (*e* to *h*), >0.1 (*g* to *j*), >0.9 (*h* to *j*).

tumours were teratocarcinomas, whereas in C57BL/6J mice, less than 10% of tumours were classified as teratocarcinomas. The proportion of teratocarcinomas in C3H/HeJ mice was significantly higher than in nude and TIR recipients. In addition, all embryo-derived tumours in nude and TIR recipients were considerably smaller than those in control mice. Tumours as well as the adjacent kidney parenchyma of both nude and TIR animals often contained aggregates of small lymphocytes.

Our results show that the removal or absence of the T-cell immune system in recipients of embryonic grafts not only does not stimulate the development of teratocarcinomas but apparently inhibits teratocarcinogenesis. Two hypotheses could explain these observations. First, in agreement with the immunostimulation theory of Prehn³, one could postulate that an interaction between the transplanted embryo and the T-cell immune system is necessary for teratocarcinogenesis. TIR mice are not completely devoid of T-cell functions⁴, but the number of T cells they have might be insufficient for immunostimulation. The alternate hypothesis is that the development of teratocarcinomas was inhibited by natural killer cells and/or macrophages. Nude mice have high levels of natural killer-cell activity⁵ and, as such cells are derived from bone marrow⁶, their activity is probably also restored in TIR mice. Depletion of T lymphocytes increases resistance to syngeneic tumours, and activated macrophages have been suggested as cells mediating such resistance⁷. The presence of lymphocytic infiltrates in the embryo-derived tumours obtained in nude and TIR mice supports the latter hypothesis. Because of the unique nature of these embryo-derived tumours we have been able to show that the T-cell status of the recipient mice affects the nature of the tumour (benign or malignant) in a situation where the incidence of tumours is unaffected.

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1. Damjanov, I. & Solter, D. *Curr. Topics Path.* **59**, 69–130 (1974).
2. Solter, D., Damjanov, I. & Koprowski, H. in *The Early Development of Mammals* (eds Balls, M. & Wild, A. E.) 243–264 (Cambridge University Press, 1975).
3. Prehn, R. T. *J. natn. Cancer Inst.* **59**, 1043–1049 (1977).
4. Gorczynski, R. M. & MacRae, S. *Immunology* **33**, 697–712 (1977).
5. Herberman, R. B. & Holden, H. T. *Adv. Cancer Res.* **27**, 305–377 (1978).
6. Haller, O. & Wigzell, H. *J. Immun.* **118**, 1503–1506 (1977).
7. Woodruff, M., Dunbar, N. & Ghaffar, A. *Proc. R. Soc. B* **184**, 97–102 (1973).

Antigen-specific suppressive factor produced by a transplantable I-J bearing T-cell hybridoma

THE demonstration of a definite antigen-binding specificity possessed by suppressor T cells and suppressor factors^{1–6} has provided an important strategy to determine the T-cell receptor for antigen. Although the gene coding for the determinant on the suppressor molecule has been unambiguously mapped in the I-J subregion of the mouse major histocompatibility complex (MHC)^{3,7}, the chemistry and the mode of action of the active molecule have been only poorly analysed—probably because of the difficulty in collecting sufficient materials from suppressor T cells for each studies. The establishment of T-cell hybridomas with specific suppressor function would provide an ideal tool to analyse the suppressor molecule and the mechanism of its action. We have been successful in obtaining a number of hybrid cell lines continuously secreting molecules having both antigen-specific suppressor function and I-J determinants. High frequency of fused cells with I-J determinants was reproducibly made by the combination of two procedures; the method to

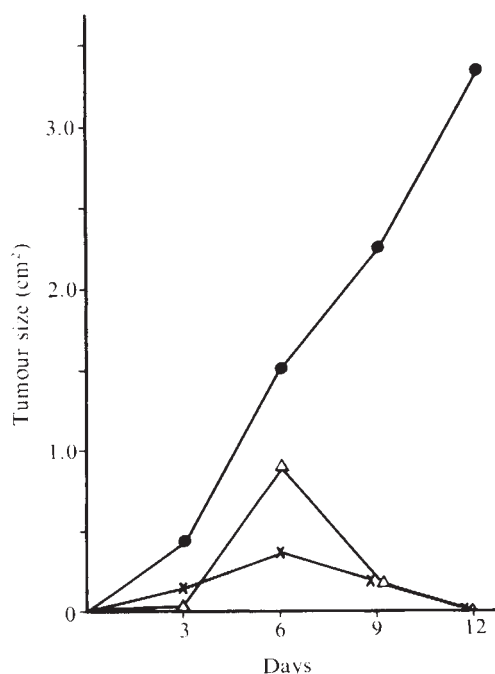


Fig. 1 Growth of I-J^b positive hybridoma 9F181a in F₁ mice but not in mice with parental haplotypes. 2×10^6 9F181a cells in 0.1 ml were inoculated into C3H (H-2^k) (X), C57BL/6 (H-2^b) (Δ) or (C3H x C57BL/6)F₁ (H-2^{b/k}) mice (●). Tumour size was measured on the day indicated and expressed as the mean tumour area, being represented by the product of two perpendicular diameters. Each group consisted of four animals.

Table 1 Antigen-specific suppressive effect of secreted or extracted materials from the I-J^{b+} hybridoma 9F181a on the *in vitro* secondary anti-DNP IgG PFC responses

	Suppressor factor	Dose	Anti-DNP IgG PFC response to	
			DNP-EA	DNP-KLH
Expt A	Not added	—	1,733 ± 125	6,295 ± 1194
	Extract of BW5147	3 × 10 ⁵ cells	1,762 ± 300	5,371 ± 1194
	Extract of 9F181a	3 × 10 ⁵ cells	1,697 ± 691	417 ± 107
Expt B	Not added	—	1,520 ± 274	1,492 ± 72
	Ascites of 9F181a-bearing mice	50 µl	1,521 ± 290	213 ± 73
		10 µl	1,307 ± 299	149 ± 88
		1 µl	1,379 ± 89	625 ± 368
		0.1 µl	1,648 ± 494	1,762 ± 137

4 × 10⁶ spleen cells from C57BL/6 mice primed with 100 µg of DNP-KLH or DNP-EA and 1 × 10⁹ pertussis vaccine 4 weeks before culture in Falcon 3008 plastic plate in 1 ml of RPMI-1640 (GIBCO) enriched with 10% fetal calf serum in the presence of 0.1 µg of antigen and 2 × 10⁻⁵ M 2-mercaptoethanol. The secreted or extracted materials from the hybridoma 9F181a were added to the culture at the start of cultivation. The culture was maintained in humidified atmosphere of 5% CO₂ in air at 37 °C. Anti-DNP IgM and IgG PFC were assayed on day 5 of culture by Cunningham and Szenberg as described previously². Expt A: 100 µl of the extract corresponding to 3 × 10⁵ BW5147 cells or hybridoma 9F181a cells was added to the appropriate group at the beginning of culture. For the preparation of the extract, cells were collected, washed three times with serum-free medium and resuspended with 0.01 M sodium phosphate-buffered saline pH 7.2 at a concentration of 1 × 10⁸ cells per ml. They were frozen in dry ice in acetone and thawed with warm water at 37 °C 10 times each. The cell-free extract was obtained by ultracentrifugation of the freezing and thawing materials at 20,000g for 1 h. Several other experiments gave similar results. Expt B: Various doses of ascites from (C57BL/6 × C3H)F₁ mice intraperitoneally implanted with 2 × 10⁷ hybridoma 9F181a cells 14 days previously were added at the beginning of the culture. Five other experiments gave similar results. Results are expressed as mean numbers of anti-DNP IgG PFC of four or five cultures ± s.d.

enrich antigen-specific Ig⁻ T cells from primed spleen cells by adsorption to and elution from antigen-coated Petri dishes, and the selection of I-J⁺ hybrids by sorting out with fluorescence activated cell sorter (FACS) after staining the fused cells with appropriate fluorescence reagents⁸. Here we report the derivation of an I-J bearing hybrid cell line, which is transplantable to H-2^{b/k} F₁ hybrid mice, secreting the antigen-specific suppressor molecule. Some physicochemical and immunochemical properties are also described.

The hybrids were made by fusion of AKR thymoma cell line BW5147, which lacks the enzyme hypoxanthine—guanine phosphoribosyl transferase (HGPRT⁻), with the suppressor T cell of C57BL/6 mice specific for keyhole limpet haemocyanin (KLH). The KLH-binding suppressor T cells were enriched by

Table 2 Immunochemical characterisation of the suppressive molecule in the secreted and extracted materials from the 9F181a hybridoma

Suppressor factor absorbed with:	Anti-DNP IgG PFC per culture	
	Ascites	Extract
Not added	1,023 ± 401	1,169 ± 114
Unabsorbed	86 ± 49	12 ± 23
KLH	1,492 ± 115	1,251 ± 233
Ascites	32 ± 64	35 ± 45
Anti-mouse Igs	96 ± 65	47 ± 56
Anti-mouse Fab	149 ± 175	59 ± 70
Anti-H-2 ^b	1,204 ± 141	1,180 ± 276
Anti-I-J ^b	1,364 ± 92	947 ± 159
Anti-I-J ^k	149 ± 74	24 ± 47

20 µl of ascites or extracted materials from the 5 × 10⁵ hybridoma cells were absorbed by passing through the following immunoabsorbents: KLH (5 mg KLH per ml beads), *Ascaris suum* extract (5 mg protein per ml beads), anti-H-2^b (C3H.Q × HTH anti-C3H.B10; 1 ml γ-globulin fraction of antiserum per ml beads), anti-I-J^b (B10.A(5R) anti-B10.A(3R); 1 ml γ-globulin fraction of antiserum per ml beads), anti-I-J^k (B10.A(3R) anti-B10.A(5R); 1 ml γ-globulin fraction of antiserum per ml beads), polyvalent rabbit anti-mouse Igs (5 mg purified anti-mouse Ig antibodies per ml beads), and rabbit anti-mouse Fab (5 mg purified anti-mouse Fab antibodies per ml beads). The beads used were Sepharose 4B. The method for preparation of immunoabsorbents was as described previously³. The absorbed materials were added to the culture of spleen cells of C57BL/6 mice primed with DNP-KLH. The assay of anti-DNP IgG PFC is the same as in Table 1.

allowing Ig⁻ spleen cells from KLH-primed mice to bind to KLH-coated Petri dishes⁹. Cells thus obtained constituted about 0.1–0.5% of the original spleen cells, but were enriched for the suppressor activity about 100-fold. The method for cell fusion used was as described elsewhere⁸. In brief, a mixture of 1 to 5 × 10⁶ enriched suppressor T cells and 1 to 50 × 10⁶ of BW5147 thymoma cells was suspended in Dulbecco's modified Eagle's medium (DMEM). The mixture was centrifuged at 400g and the pellet resuspended in 2 ml of polyethylene glycol (PEG: MW 2,000)—dimethyl sulphoxide (DMSO) solution (1 weight of PEG plus 1 volume of 15% DMSO–DMEM). Within 1 min, a further 2 ml of 50% (w/v) PEG solution without DMSO was poured into the test tube, and the reaction mixture gently stirred with a Pasteur pipette. The suspension was then gradually diluted with 16 ml of serum free DMEM and further diluted with 180 ml of DMEM containing 13% of fetal calf serum (FCS). They were then incubated at 37 °C in 5% CO₂ for 3–4 h. After incubation cells were washed four times with DMEM and cultured for 1 week in HAT medium (RPMI-1640 supplemented with 10% FCS and hypoxanthine, aminopterin, thymidine (HAT)) on Falcon plates (Falcon 3008), each well containing about 2 to 10 × 10⁴ cells in 1 ml. Cells grown in HAT medium were collected and stained with an anti-I-J^b antiserum (B10.A(5R) anti-B10.A(3R)) and fluorescein-conjugated rabbit anti-mouse Fab, both of which had been extensively preabsorbed with BW5147 cells to avoid nonspecific staining. The stained cells were then sorted by fluorescence activated cell sorter, FACS II (Becton-Dickinson), and fluorescence positive cells were cloned in multi-well microplates (Falcon 3040) by limiting dilution method or single cell manipulation.

Several hybridisation experiments have been carried out, and so far seven antigen-specific suppressor T-cell lines established. The detailed properties of these cell lines will be described elsewhere. The cell line 9F181a described here displayed certain characteristics not possessed by other functional T-cell hybridomas: this line was transplantable to H-2^{b/k} F₁ mice and secreted the antigen-specific suppressor molecule having I-J determinants, thus providing an opportunity to compare the suppressor molecules secreted and extracted from the suppressor T-cell hybridoma.

The transplantability of 9F181a was studied by inoculating 2 × 10⁶ cells in 0.1 ml intracutaneously into the shaved back of C57BL/6 (H-2^b), C3H (H-2^k) and (C57BL/6 × C3H)F₁ (H-2^{b/k}) mice respectively. The active growth of the tumour as a

Table 3 Molecular weight of the suppressor factor in ascites of mice bearing 9F181a

Fraction added	Anti-DNP PFC per culture		% Suppression of IgG PFC
	IgM	IgG	
No factor	688 ± 58	2,544 ± 59	—
Fraction I	756 ± 58	2,956 ± 211	0
Fraction II	756 ± 365	2,590 ± 89	0
Fraction III	596 ± 89	2,865 ± 431	0
Fraction IV	779 ± 380	413 ± 325	84
Fraction V	668 ± 175	2,062 ± 325	19

The materials fractionated on Sephadex G-200 as indicated in Fig. 2 were concentrated by vacuum dialysis at 4 °C and tested for suppressive activity. The fractionated material at a dose corresponding to 30 µl of original ascites was added to the culture of spleen cells of C57BL/6 primed with DNP-KLH. Results are expressed as mean numbers of anti-DNP IgM and IgG PFC ± s.d. The degree of suppression was calculated as follows:

$$\% \text{ Suppression} = (1 - \text{experimental PFC/control PFC}) \times 100$$

solid form was observed in (C57BL/6 × C3H)F₁ mice, but not in parental C3H or C57BL/6 mice (Fig. 1). If the cells were transplanted intraperitoneally into F₁ mice, a large quantity of ascites was produced. This ascites contained a strong antigen-specific suppressor activity (see below). The transplantability of 9F181a is partially explained by its surface phenotypic expressions: Even 13 months after hybridisation the cell line expressed H-2 antigens of both parental cells, H-2^b, and H-2^k, together with I-J^b determinants introduced from C57BL/6 suppressor T cells.

Previous studies have firmly established that the extract from sonicated splenic T cells of KLH-primed mice can specifically suppress the *in vivo* and *in vitro* secondary IgG antibody responses against DNP-KLH^{2,10}. The active molecule involved in this suppression was shown to carry determinants coded for by genes mapped in I-J subregion of MHC³. It was therefore of interest to establish whether the ascites as well as the extract obtained from this hybridoma contains a similar molecule having I-J determinants and antigen-specific suppressor activity. Various doses of ascites obtained from (C57BL/6 × C3H)F₁ mice bearing 9F181a were added to the culture of 4 × 10⁶ DNP-KLH- or DNP-EA (hen's egg albumin: hapten coupled to irrelevant carrier)-primed C57BL/6 spleen cells which were concomitantly stimulated with the corresponding antigen (0.1 µg ml⁻¹) in the Mischell-Dutton culture system. A separate experiment was carried out to study the suppressive activity of the extract of 9F181a. The dose of extracted material corresponding to 3 × 10⁵ viable cells was added to the culture. The number of plaque forming cells producing anti-DNP IgG antibody was measured on day 5 by the method of Cunningham and Szenberg as described previously².

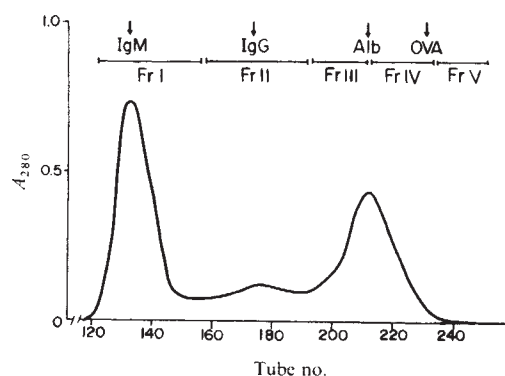


Fig. 2 Elution profile of ascites from mice bearing 9F181a after fractionation on Sephadex G-200. Two ml ascites was dialysed in 0.005 M borate buffered saline pH 8.0 for 16 h. The dialysed material was then fractionated on Sephadex G-200 column (2.5 × 90 cm × 2). Fractions (3 ml) were monitored by a spectrophotometer at 280 nm. Five fractions were separated as indicated, and were tested for suppressive activity (see Table 3). Arrows indicate the position of marker proteins (mouse IgM, mouse IgG, mouse albumin and hen's egg albumin: OVA) eluted at the identical condition. The MW of Fr. I is >200,000; Fr. II, 100,000–200,000; Fr. III, 70,000–98,000; Fr. IV, 42,000–68,000; Fr. V, 30,000–40,000.

The results in Table 1 show that addition of 1–50 µl of ascites to the cultured spleen cells suppressed anti-DNP IgG antibody response to DNP-KLH but not to DNP-EA. Even 10 µl of ascites showed a maximal suppressive effect, and 1 µl was enough to show a significant suppression. The extracted material from 9F181a also showed the same carrier specific suppressor activity, and the same dose of the extract from parental BW5147 had no effect on the response against both antigens. IgM antibody-forming cells to DNP-KLH which comprised 20 to 30% of total antibody secreting cells were not suppressed by both extracted and secreted materials (data not shown).

Immunochemical properties of the hybridoma-derived suppressor factors were studied by absorption with immunoadsorbents composed of specific antigen (KLH), irrelevant antigen (*Ascaris* extract), anti-H-2, anti-I-J, polyvalent anti-mouse Igs and anti-mouse Fab antibodies. As shown in Table 2, the suppressive activity of both ascites and extract was completely removed by KLH, anti-H-2^b and anti-I-J^b, but not by *Ascaris* extract, anti-I-J^k, polyvalent antimouse Igs and anti-mouse Fab. These results indicate that the suppressor molecules both secreted and extracted from 9F181a have an antigen-binding site and I-J coded determinants.

The molecular size of the hybridoma-derived suppressor molecule was investigated. Figure 2 shows the elution profile of

Table 4 Suppressive effect of extracted and secreted materials from 9F181a on the response of C57BL/6 and (C57BL/6 × C3H)F₁ but not of C3H mice

Suppressor factor	Dose	Anti-DNP IgG PFC per culture		
		C57BL/6	(C57BL/6 × C3H)F ₁	C3H
Not added	—	1,854 ± 288	3,424 ± 345	6,160 ± 725
Extract of BW5147	3 × 10 ⁵ cells	2,054 ± 245	3,194 ± 435	5,025 ± 1,109
Extract of 9F181a	3 × 10 ⁵ cells	134 ± 114	586 ± 122	5,382 ± 530
Ascites of 9F181a-bearing mice	20 µl	682 ± 272	1,008 ± 568	6,543 ± 1,264

Extract equivalent to 3 × 10⁵ BW5147 or hybridoma 9F181a cells and 20 µl of ascites was added to the culture of spleen cells of C57BL/6 (H-2^b), C3H(H-2^k) and (C57BL/6 × C3H)F₁ (H-2^{b/k}) mice immunised with DNP-KLH and pertussis vaccine to test the requirement of H-2 histocompatibility for effective suppression. The numbers of anti-DNP IgG PFC after 5 days of culture with and without the factor were compared. Assay of anti-DNP IgG PFC as in Table 1.

ascites on Sephadex G-200. The fractionated materials as indicated in Fig. 2 were tested for their suppressive activity. This was present in fraction IV (Table 3). The MW of fraction IV was between 42,000 and 68,000—similar to that of previously reported suppressor factor from splenic T cells².

We have previously demonstrated that the suppressor T-cell factor from primed mice was able to suppress only the responses of H-2 histocompatible strains¹¹. Thus, we attempted to examine whether or not such a genetic restriction exists in the effect of the suppressor factor derived from the hybridoma 9F181a. As the cell line was made by fusion of BW5147 (H-2^k) and C57BL/6 (H-2^b) suppressor T cells, the secreted and extracted materials from 9F181a were tested for their activity to suppress the response mounted by spleen cells of parental haplotypes, H-2^k and H-2^b and of their F₁ hybrid (H-2^{b/k}). The results in Table 4 show that the responses of C57BL/6 and (C57BL/6 × C3H)F₁ were suppressed by both secreted and extracted materials, whereas that of C3H (H-2^k) was not. This result is consistent with the fact that I-J^b but not I-J^k is phenotypically expressed on 9F181a. The finding that the putatively homogeneous suppressor factor derived from the hybridoma had this strict H-2 restriction supports our previous postulate that the I-J bearing factor can interact with other types of lymphoid cells only if they share an identical I-J subregion¹¹.

A T-cell hybridoma with antigen-specific suppressor activity has recently been reported by Kontiainen *et al.*¹². The suppressive factor from their hybridoma was shown to suppress mainly the primary IgM antibody response, while the secondary IgG antibody response was less affected. The antigen-specific suppressor factor from our hybridoma preferentially suppressed secondary IgG response without measurable suppression of the IgM antibody response. The same was true for the hybridoma-derived factors reported by Taniguchi and Miller⁸ in the *in vivo* suppression of the secondary antibody response to other protein antigen, in which antigen-specific hybridoma derived factors could suppress only the IgG antibody response. Whether such a difference would be inherent to the structure of the factors is not yet known.

The establishment of T-cell hybridoma cell lines continuously producing homogeneous materials with antigen-specific activity would make possible a more exact analysis of the molecule and its mode of action. The transplantable hybridomas secreting a large quantity of the active molecules in ascites or in the culture supernatant would be of particular value in the study of the chemistry of the molecule.

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1. Tada, T., Taniguchi, M. & Takemori, T. *Transplant Rev.* **26**, 106–129 (1975).
2. Taniguchi, M., Hayakawa, K. & Tada, T. *J. Immun.* **116**, 542–548 (1976).
3. Tada, T., Taniguchi, M. & David, C. S., *J. exp. Med.* **144**, 713–725 (1976).
4. Thèze, J., Kapp, J. A. & Benacerraf, B., *J. exp. Med.* **145**, 839–856 (1977).
5. Taniguchi, M. & Miller, J. F. A. P., *J. Immun.* **120**, 21–26 (1978).
6. Kontiainen, S. & Feldmann, M., *Eur. J. Immun.* **7**, 310–314 (1977).
7. Murphy, D. B., Herzenberg, L. A., Okumura, K., Herzenberg, L. A. & McDevitt, H. O. *J. exp. Med.* **144**, 699–712 (1976).
8. Taniguchi, M. & Miller, J. F. A. P. *J. exp. Med.* **148**, 373–382 (1978).
9. Taniguchi, M. & Miller, J. F. A. P. *J. exp. Med.* **146**, 1450–1454 (1977).
10. Takemori, T. & Tada, T. *J. exp. Med.* **142**, 1241–1253 (1975).
11. Taniguchi, M., Tada, T. & Tokuhisa, T. *J. exp. Med.* **144**, 20–31 (1976).
12. Kontiainen, S. *et al. Nature* **274**, 477–480 (1978).

Interferon enhances the excitability of cultured neurones

INTERFERON has varied biological effects on cells^{1,2} and induces modifications of the cell surface both *in vitro*^{3–6} and *in vivo*⁷. The particular capacity of the neuronal membrane to generate propagated action potentials (well retained in culture⁸) makes neurones excellent material for the study of substances that affect the cell surface. We have therefore determined the effect of interferon on the electrical activity of nerve cells. Most of the experiments reported here were carried out on the cat cerebral and cerebellar cortex, because the electrical patterns of these structures have been well defined *in vitro*^{8,9}. Furthermore, as human leukocyte interferon exhibits a high degree of antiviral activity on cat cells¹⁰, we were able to study the effect of highly purified interferon preparations on the spontaneous and evoked electrical activity of these neurones. In other experiments, we studied the effect of rat interferon on rat neurones in culture. Our results, which show that human and rat interferon specifically enhance the excitability of cat and rat nerve cells, respectively, may have implications for the therapeutic use of interferon as well as for the understanding of some of the manifestations of viral diseases.

Explants of cerebral and cerebellar cortex from newborn kittens or rats were cultivated in Maximow assemblies at 35.5 °C in a drop of nutrient medium containing 40% fetal calf serum⁸. After 3 weeks, the culture was transferred into a moist chamber and fixed on the stage of an inverted phase contrast microscope.

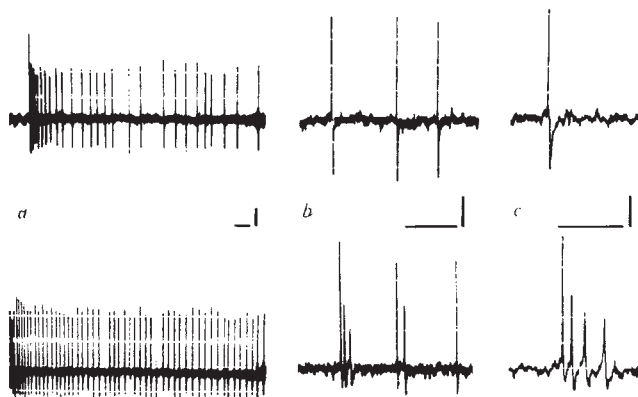


Fig. 1 Effect of human leukocyte interferon on the spontaneous activity of a Purkinje cell (kitten cerebellar cortex: 43 d in culture). The upper (before interferon) and lower tracings (4 h after interferon) are recorded from the same cell for different sweep rates (*a*, *b*, *c*). Note that interferon increases the spike frequency and the duration of the burst (*a*). *b* and *c* (faster sweeps) show the tendency of the interferon-treated neurone to fire repeatedly (2–4 successive action potentials) compared with the single spike obtained from the same neurone before interferon. Horizontal bars, 100 ms; vertical bars, 1 mV; negativity downwards.

Under visual control, the two recording electrodes (glass micropipettes filled with 3 M KCl with a resistance of about 30 MΩ) were placed against the neuronal perikarya to record extracellularly the electrical activity of two neurones (or groups of neurones) for several hours. Focal electrical stimuli (15 V, 0.2–1 ms) were applied through a pair of electrodes (filled with isotonic saline, with the same resistance, 0.5–1 MΩ) which could be placed successively in various sites of the explant. The responses were amplified by a differential field effect transistor preamplifier (a.c. recording-time constant 250 ms), and stored on tape.

Table 1 Statistical analysis of the latency of 12 different cortical responses before and at least 1 h after addition of human leukocyte interferon

Stimulation site	No. of responses before and after interferon	Mean latency (ms $\pm$ s.d.)
1	Before 32	40 $\pm$ 9.4
	After 15	17 $\pm$ 4.7*
2	Before 30	36 $\pm$ 6.5
	After 16	29 $\pm$ 5.5†
3	Before 17	38 $\pm$ 5.5
	After 15	23 $\pm$ 7.4*
4	Before 31	16 $\pm$ 3.1
	After 21	10 $\pm$ 1.7*
5	Before 15	34 $\pm$ 5.9
	After 15	14 $\pm$ 7.1*
6	Before 15	67 $\pm$ 9.8
	After 15	30 $\pm$ 4.8*
7	Before 18	42 $\pm$ 7.1
	After 30	24 $\pm$ 4.7*
8	Before 15	109 $\pm$ 17.2
	After 16	75 $\pm$ 10.3*
9	Before 29	57 $\pm$ 15.5
	After 29	15 $\pm$ 8.5*
10	Before 16	68 $\pm$ 17.0
	After 15	12 $\pm$ 2.5*
11	Before 16	33 $\pm$ 5.6
	After 16	22 $\pm$ 5.2*
12	Before 25	33 $\pm$ 10.1
	After 25	13 $\pm$ 5.4*

Results were from three different kitten cerebral explants. Analysis by *t* test.

* Highly significant, $P < 10^{-9}$.

† $P < 0.0001$.

Crude human leukocyte interferon prepared as previously described¹¹ was purified over 3,000-fold and had a specific activity of 10^7 units per mg protein. It had a titre of 5.12×10^{-5} as assayed (using vesicular stomatitis virus) on both human diploid fibroblasts and cultures of kidney from the same kitten whose brain cortical cells were used in the experiment. A control human leukocyte preparation of approximately the same protein content (4 mg ml^{-1}) was prepared as previously described¹¹. Rat cell culture interferon prepared according to the techniques used in the production of mouse interferon¹² had a titre of 8×10^{-5} on rat fibroblasts and a specific activity of 8×10^5 units per mg protein. Because of the inherent complexity of the system it was only feasible to use a single interferon concentration for each species. We chose 10^4 human leukocyte interferon units and 8×10^3 rat cell culture interferon units.

When human leukocyte interferon was added to the culture medium of an explant from kitten cerebellar cortex there was an increase in the frequency of the spontaneous unit discharges and a tendency for individual neurones to fire repeatedly (Fig. 1). These changes were first observed after approximately 30 min and became more marked after several hours. In explants of kitten cerebral cortex the spike and slow wave pattern, which was usually observed at a frequency of about 1 per min, occurred every 30–40 s after the addition of interferon, and often showed a tendency to repeated firing. Rat interferon induced comparable effects on the neurones from newborn rat cerebellar and cerebral cortex. It is important to emphasise that although interferon induced quantitative modifications, it did not modify the basic spontaneous electrical pattern characteristic of these neurones⁸ (the cerebellar bursts¹³ and the cerebral spike and wave^{8,14}).

As interferon enhanced the spontaneous electrical activity of cat and rat neurones, we studied the effect of human interferon on the evoked electrical activity of cat neurones. In the experiments, the responses were plotted for different stimulating sites before and at various intervals after the addition of interferon. The general pattern of the evoked responses was similar to that characteristic of spontaneous activity and was reproducible for several hours. As in the experiments on spontaneous electrical

activity, an effect of interferon was first observed at approximately 30 min and persisted for the whole 4–5-h recording session.

The most important effect of interferon was a marked shortening of the latency between stimulation and the initial response (Fig. 2a, b). This decrease in latency was observed for all the stimulating sites at both recording electrodes, and the statistical analysis of these results is presented in Table 1. Interferon also lowered the threshold to stimulation. Thus, for a given intensity, a single shock (0.7–1.0 ms) evoked a single wave before interferon (Fig. 2c, upper tracings), whereas a similar but repetitive pattern was observed after the addition of interferon (Fig. 2c, lower tracings). Furthermore, the duration of the shock necessary to elicit a single response could be decreased from 0.7 ms to 0.3–0.2 ms after the addition of interferon. Lastly, when treated neurones were subjected to a repetitive stimulation, they were able to respond at a higher rate (6–8 potentials

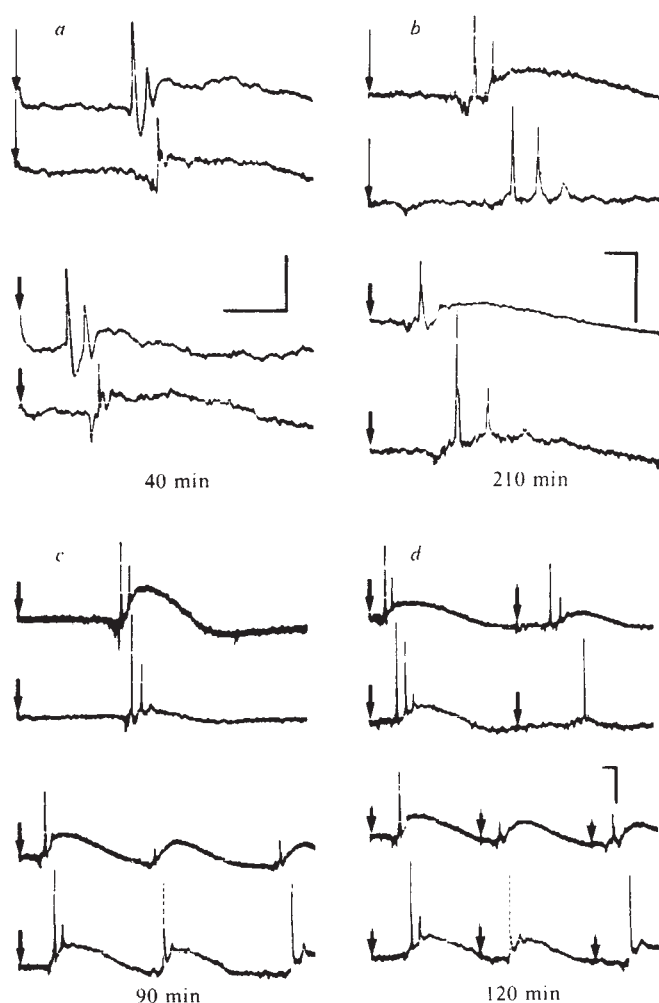


Fig. 2 Patterns of evoked electrical activity in cultures of kitten motor cortex ($a = 46$ d) and hippocampus ($b, c, d, 58$ d) before (two upper tracings in each panel) and after (two lower tracings in each panel) treatment with 10^4 units of human leukocyte interferon. *a* and *b* illustrate the shortening of the latency after addition of interferon (see time in minutes in lower tracings) for the same stimulating site and for the same neurones. In *c*, a single shock of 0.7 ms evoked a single spike and wave from both neurones before interferon, and three successive spikes and waves 90 min after the addition of interferon. Note that the latency of the repetitive firing in the lower two tracings is considerably shorter than that of the single response shown in the two upper tracings. In *d*, two shocks at intervals of 250 ms evoked only two responses before interferon, whereas 2 h after the addition of interferon, three shocks at intervals of 175 ms still evoked three successive responses with a constant shortened latency. Note in all panels that the polarity and amplitude of the waves and the order of firing of both neurones remain unchanged after interferon. Horizontal bars, 20 ms; vertical bars 1.5 mV; negativity downwards. The length of the arrows indicating stimulation is directly proportional to the intensity of the current.

per s instead of 4–5 potentials per s) (Fig. 2d). It is interesting that interferon did not, however, increase the amplitude or modify the polarity of the electrical potentials.

We must stress that neither human nor rat interferon preparations was toxic for neurones in culture. The behaviour of interferon-treated cells did not change during the several hours of observation, and after staining (with Bodian silver impregnation) the neurofibrillar nets had the same normal morphological appearance as those in control cultures. Furthermore, when comparable amounts of human and rat interferon were added to fresh cat and rat explants, these cultures could be maintained for 1 month with interferon without any detectable damage.

The evidence that interferon was responsible for these effects may be summarised as follows. Only human leukocyte interferon, which exerts antiviral activity on cat cells, enhanced the spontaneous activity and evoked responses of cat cerebellar and cerebral cortex: a control leukocyte preparation and rat cell culture interferon did not exert these effects. Likewise, rat cell culture interferon, but not control preparations, enhanced the electrical activity of rat neurones.

An enhanced excitability of cerebral and cerebellar neurones in culture has also been observed using bicuculline¹⁵, picrotoxin¹⁵ and strychnine^{14,16}. Some of the effects of these substances (shortened latency, lowered threshold, tendency to repetitiveness) are comparable to those we have described for interferon. Some striking differences do exist, however, between the effects of interferon on neurones and those reported for other pharmacological agents¹⁷. (1) Interferon treatment did not increase the amplitude or reverse the polarity of the cortical waves (as do most of the other excitatory substances). (2) The effect of interferon was seen after 30 min and lasted several hours. (Most other substances induce an immediate effect which lasts only a few minutes.)

Our data do not permit us to determine whether the enhanced excitability results from a direct effect on the neurone (modification of transmembrane ionic exchanges) or at the level of the synapse (facilitation of excitatory or blocking of inhibitory synapses). We believe this to be the first report of an effect of interferon on the function of nerve cells in culture. The nature of this effect is consistent with other known actions of interferon on cells, that is, enhancement of a particular cell function in the absence of cell toxicity^{1,2}. Although most of the effects of interferon require its incubation with cells for periods over 12 h, some early effects of interferon suggestive of modification of the cell surface have been observed within several hours; these include changes in thymidine transport¹⁸, release of labelled uridine¹⁹, and enhanced cytotoxicity of sensitised T lymphocytes²⁰ or natural killer cells²¹. However, the enhancement by interferon of the excitability of neurones within 30 min seems to be one of the earliest effects yet described.

Our observations may, however, be of more general interest. If the interferon-induced enhancement of neurone excitability in culture also occurs *in vivo*, our results may be relevant to some of the untoward manifestations of viral diseases.

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- Gresser, I. *Cell Immun.* **34**, 406–415 (1977).
- Gresser, I. & Tovey, M. G. *Biochim. biophys. Acta* **516**, 231–247 (1978).
- Lindahl, P., Leary, P. & Gresser, I. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2785–2788 (1973).
- Huet, C., Gresser, I., Bandu, M-T. & Lindahl, P. *Proc. Soc. exp. Biol. Med.* **147**, 52–57 (1974).
- Knight, E. Jr & Korant, B. D. *Biochem. biophys. Res. Commun.* **74**, 707–713 (1977).
- Chang, E. H., Jay, F. T. & Friedman, R. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1859–1863 (1978).
- Lindahl, P., Gresser, I., Leary, P. & Tovey, M. G. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1284–1287 (1976).
- Calvet, M-C. *Brain Res.* **69**, 281–295 (1974).
- Calvet, M-C. & Lepault, A. M. *Expl Brain Res.* **23**, 249–258 (1975).
- Desmyter, J. & Stewart, W. E. II. *Virology* **70**, 451–458 (1976).
- Mogensen, K. E. & Cantell, K. *Pharmac. Ther.* **C1**, 369–381 (1977).
- Tovey, M. G., Begon-Lours, J. & Gresser, I. *Proc. Soc. exp. Biol. Med.* **146**, 809–815 (1974).
- Leiman, A. L. & Seil, F. J. *Expl Neurol.* **40**, 748–759 (1973).
- Crain, S. M. & Bornstein, M. B. *Expl Neurol.* **10**, 425–450 (1964).
- Gähwiler, B. H. *Brain Res.* **99**, 85–95 (1975).
- Gähwiler, B. H. *J. Neurobiol.* **7**, 97–107 (1976).
- Crain, S. M. *Neurophysiologic Studies in Tissue Culture* (Raven, New York, 1976).
- Brouty-Boyd, D. & Tovey, M. G. *Intervirology* **9**, 243–252 (1977).
- Degré, K. & Hovig, T. *Acta path. microbiol. scand.* **B84**, 347–358 (1976).
- Lindahl, P., Leary, P. & Gresser, I. *Proc. natn. Acad. Sci. U.S.A.* **69**, 721–725 (1972).
- Gidlund, M., Orn, A., Wiggzell, H., Senik, A. & Gresser, I. *Nature* **277**, 759–761 (1978).

Stimulant effects of enkephalin microinjection into the dopaminergic A10 area

THE overt behaviour of various species is affected in different ways by opiate drugs. Mice and cats show behavioural stimulation following a systemic morphine injection whereas rats and dogs generally exhibit decreased behavioural activity^{1–4}, although in certain conditions, stimulant effects can also be seen in the latter species^{4,5}. Tatum *et al.* suggested that there are several distinct sites of action of opiate drugs in brain and that the relative dominance of one system over another determines whether the stimulant or depressant effects predominate⁴. Indeed, direct microinjections of morphine into the ventral tegmental area (VTA) have been shown to result in facilitation of self-stimulation behaviour whereas similar injections into the periaqueductal gray matter cause only an attenuation of this behaviour⁶. The discovery of endogenous ligands for opiate receptors in the brain⁷ has focused attention on the role of these peptides in various aspects of behaviour. Their analgesic properties following intraventricular and intracerebral injection are well documented⁸. Recently, it has been reported that infusion of α -endorphin into the substantia nigra induced stereotyped behaviour⁹, whereas local injections into the nucleus accumbens of the long-acting synthetic enkephalin analogue, [D-Ala²]-Met⁵-enkephalinamide (AME), produced an increase in locomotor activity¹⁰. We now report that infusion of AME (0.03–2.0 μ g) bilaterally into the dopaminergic (DA) A10 area of the VTA induces a short-latency behavioural stimulant effect reminiscent of effects produced by stimulation of the mesolimbic DA pathway¹¹. This effect is antagonised by pretreatment with the opiate receptor blocker naloxone.

Twenty-seven male Wistar rats, weighing 300–320 g, were anaesthetised with Nembutal (50 mg per kg), and two stainless-steel guide cannulae (23 gauge) were implanted stereotactically into an area 0.5 mm dorsal to the ventral tegmentum. Cannulae placements were confirmed histologically; the correct loci, quantified according to the coordinate system of König and Klippel, averaged $A = 2.1 \pm 0.3$, $L = 0.9 \pm 0.2$, and $DV = -2.6 \pm 0.5$. Behavioural testing began 14 d after surgery.

Cannulae were sealed and kept patent by insertion of a 30-gauge inner stainless-steel stylet which protruded 0.5 mm beyond the outer guide. Microinjections were made by replacing each inner stylet with a 30-gauge needle attached to a microsyringe by polyethylene tubing. A volume of 0.5 μ l was injected into each cannula over a 30-s period. The rats received injections of 2.0, 1.0, 0.12 or 0.03 μ g of AME or the vehicle (0.9% NaCl) on each side of the brain. Subjects were adapted to circular photocell cages for 2 h before injection and activity was measured for an additional 120 min after infusions into the

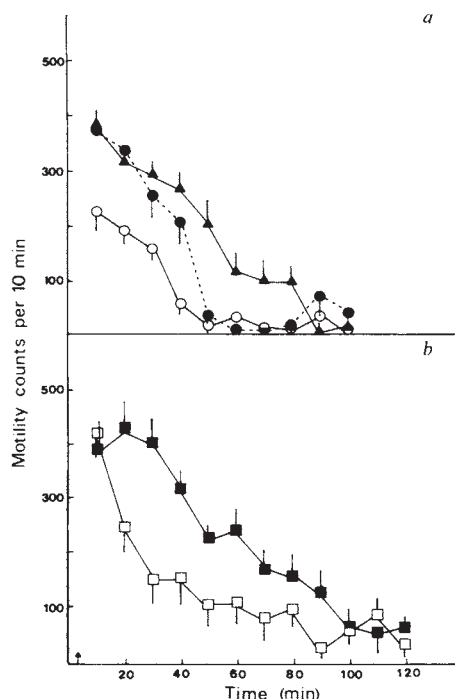


Fig. 1 Motility scores after microinjection of D-Ala²-Met⁵-enkephalinamide (AME, Calbiochem) or NaCl (0.9%) into the VTA. Ordinate: time in minutes; abscissa: number of times light beam interrupted per 10-min period (data presented are $\bar{x} \pm$ s.e.m.). *a*, Δ , $2 \times 0.12 \mu\text{g}$ AME ($n = 8$); $\bullet$, $2 \times 0.03 \mu\text{g}$ AME ($n = 8$). Total scores of both AME groups are significantly different from mean total scores of subjects injected with NaCl, $\circ$. ($P < 0.001$; 2-tailed t -tests.) *b*, $\blacksquare$, $2 \times 1 \mu\text{g}$ AME + NaCl (1 ml per kg, i.p.); $\square$, $2 \times 1 \mu\text{g}$ AME + naloxone (1.2 mg per kg in 1 ml per kg NaCl, i.p.). Mean total motility scores for the two groups are significantly different ($P < 0.02$, 1-tailed t -test). Arrow indicates injection. Whole-body locomotor activity was measured in circular photocell cages (60 cm diameter, BRS/LVE). In those animals receiving two drug injections, the injection sequence was balanced within the group and spaced at 48-h intervals. One day before the second injection $1 \mu\text{l}$ of 0.9% NaCl was infused through the cannulae to reduce possible misleading effects of residual drug deposits. Measurements were made during the light phase of the rat's diurnal cycle.

VTA. On one test, the subjects were pretreated with naloxone HCl (1.2 mg per kg intraperitoneally (i.p.)) immediately before bilateral infusions of $1 \mu\text{g}$ AME.

Figure 1 shows that bilateral injections of AME, in doses of $2 \times 1 \mu\text{g}$ ($n = 11$), $2 \times 0.12 \mu\text{g}$ ($n = 8$) or $2 \times 0.03 \mu\text{g}$ ($n = 8$), produced a clearcut increase in locomotor activity in all animals. This effect was evident in the first 10 min post-infusion and lasted up to 90 min. The mean total number of photocell counts for the $2 \times 1 \mu\text{g}$ dose was $2,656 \pm 367$ (s.e.m.) interruptions in 120 min. Seven additional animals were tested at the high dose of $2 \times 2 \mu\text{g}$ AME, which also led to a significant increase in the motility score ($2,781 \pm 300$ over 120 min). However, this score was not significantly different from that obtained with the $2 \times 1 \mu\text{g}$ dose and therefore the data were not included in Fig. 1. Significant increases in motility were observed in the groups receiving $0.12 \mu\text{g}$ and $0.03 \mu\text{g}$, with mean total counts of $1,930 \pm 163$ and $1,513 \pm 117$ per 120 min, respectively. Pretreatment with naloxone effectively antagonised the effects produced by $2 \times 1 \mu\text{g}$ AME (Fig. 1*b*). The activity score after naloxone was $1,616 \pm 234$ over 120 min, which was significantly less than the score obtained with a control dose of $2 \times 1 \mu\text{g}$ ($P < 0.02$ 1-tailed independent t test) but still above the score obtained after control infusions of NaCl ($\bar{x} = 828 \pm 68$ in 120 min $n = 16$).

The behavioural activation was defined more precisely by time sampling the behaviour of 11 rats according to a standard ethogram. The behavioural data indicated that the categories of

walking, rearing and sniffing were increased significantly only in the AME-treated group, at the expense of sleeping, sitting or lying quietly on the floor (Table 1). There was no indication of stereotyped behaviour such as repetitive head movements or prolonged sniffing on the same spot.

These results clearly indicate a behavioural stimulant effect after intracerebral infusions of AME into the VTA. No initial depressant effect was observed in any animal at any of the doses used. Although a significant stimulant effect was obtained with the lowest dose, there was evidence of a dose-response relationship, as the level of activity produced by this dose was significantly lower than that seen after the dose of $2 \times 1 \mu\text{g}$ ($P < 0.005$, 1-tailed t test).

Increased behavioural activity has been reported after microinjection of D-Ala into the nucleus accumbens¹⁰ and thalamus¹². However, the effects observed in the present study were obtained at much lower doses (0.03 – $2.0 \mu\text{g}$ compared with $10 \mu\text{g}$) and at significantly shorter latencies. This short-latency effect agrees with earlier observations of facilitation of self-stimulation behaviour following microinjection of morphine ($0.5 \mu\text{g}$) into the same area of the brain⁶. The antagonism of the behavioural stimulant effect of AME by naloxone is particularly important as it strongly suggests that the effect is mediated by opiate receptors.

Recent immunohistofluorescence data have identified enkephalin-positive perikarya in the interpeduncular nucleus, and enkephalin-containing nerve fibres in the VTA¹³. Thus, the present findings and the results of a previous localisation study with morphine⁶ suggest that the stimulant effects of AME are probably mediated by its action within the VTA or interpeduncular nucleus. The fact that infusions dorsal to this area were less effective (unpublished observations) is also consistent with this interpretation. It is important to recognise the difficulties in the precise localisation of intracerebral infusions. Thus, as the region of stimulation is inferred from the cannulae placements, the possible contribution of the adjacent substantia nigra cannot be ruled out. However, the behavioural data point to the involvement of the VTA in the effects reported here, as the increased locomotor activity and

Table 1 Effect on behaviour of $2 \times 2 \text{ g}$ D-Ala²-Met⁵-enkephalinamide (AME)

Behaviour	AME	Control	Statistic Z
Walking	2.5 (1–9)	0 (0–3)	2.09*
Rearing	6.5 (3–12)	2 (2–3)	
Sniffing	34.5 (32–39)	24 (20–33)	
Explorative combined	46 (39–54)	30 (22–39)	2.18*
Teeth chattering + 'freezing'	0 (0–1)	0 (0–1)	2.18*
Sitting	1 (0–4)	6 (3–21)	
Lying	0 (0–4)	6 (1–15)	
Sleeping	0 (0–1)	2 (0–13)	
Passive combined	2 (0–8)	22 (9–31)	
Burying	1 (0–6)	0 (0–1)	
Picking up bedding material	0 (0–2)	0 (0)	
Gnawing	2 (0–18)	0 (0–15)	
'Wet dog' shivering	0 (0)	0 (0–1)	
Grooming	3.5 (0–10)	6 (0–23)	

For observations, the rats were placed in circular glass chambers (30 cm diameter) with bedding material distributed evenly over the floor, and given 1 h to adapt to this environment. The figures represent the number of occasions on which the specified behaviour was observed in the period 10–52 min after injection (median values and ranges are given for AME ($n = 6$) and control ($n = 5$) groups, respectively). Observations were made every 10 s during the first 2 min of each 10-min period (total: 60 samples per rat in 52-min test period). Statistical estimates were obtained with a Wilcoxon test (2-tailed). Walking + sniffing scored as walking; rearing + sniffing scored as rearing; sniffing + sitting or lying scored as sniffing.

* $P \leq 0.05$.

exploration elicited by AME are reminiscent of the intense exploratory activity seen after microinjections of dopamine into the nucleus accumbens¹¹.

The nucleus accumbens is one of the important projection areas of the DA cell bodies in the VTA¹⁴ and there is strong evidence for the involvement of this mesolimbic DA pathway in the stimulant effects of amphetamine and other related compounds¹⁵. Although the role of dopamine in the locomotor effects produced by AME injections into the nucleus accumbens is uncertain¹⁰, there is evidence that opiates may produce behavioural excitation by activating DA neurones^{1,2}. Recent electrophysiological data also support this contention¹⁶. Thus, it is tempting to speculate that the mesocorticolimbic DA system may mediate some of the locomotor stimulant effects of enkephalin in particular and opiates in general. The mesolimbic DA pathway has also been implicated in positive reinforcement¹⁷ and motivation¹⁸ and it remains to be determined whether this pathway might mediate the reinforcing properties of enkephalin¹⁹. If such a relationship were confirmed, it would have important implications for understanding the addictive properties of opiates.

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- Caroll, B. J. & Sharp, P. T. *Br. J. Pharmac.* **46**, 124–139 (1972).
- Cools, A. R., Janssen, H. J. & Broekkamp, C. L. E. *Archs int. Pharmacodyn.* **210**, 163–174 (1974).
- Kuschinsky, K. & Hornykiewicz, O. *Eur. J. Pharmac.* **19**, 119–122 (1972).
- Tatum, A. L., Seevers, M. H. & Collins, K. H. *J. Pharmac. exp. Ther.* **36**, 447–475 (1929).
- Ayhan, I. H. & Randrup, A. *Psychopharmacology* **29**, 317–328 (1973).
- Broekkamp, C. L. E., van Dongen, P. A. M. & van Rossum, J. M. in *Psychobiology of the Striatum* (eds Cools, A. R., Lohman, A. H. M. & van den Bercken, J. H. L.) 61–72 (North-Holland, Amsterdam, 1977).
- Hughes, J., Smith, T., Morgan, B. & Fothergill, L. *Life Sci.* **16**, 1753–1758 (1975).
- Yask, T. L. & Rudy, T. A. *Pain* **4**, 299–359 (1978).
- Iwamoto, E. T. & Way, E. L. *J. Pharmac. exp. Ther.* **203**, 347–359 (1977).
- Pert, A. & Sivit, C. *Nature* **265**, 645–647 (1977).
- Pijnenburg, A. J. J. & van Rossum, J. M. *J. Pharm. Pharmac.* **25**, 1003–1005 (1973).
- Bergmann, F. *et al. Experientia* **33**, 217 (1977).
- Uhl, G. R., Goodman, R. R., Kuhar, M. J. & Snyder, S. H. in *Advances in Biochemistry and Psychopharmacology* (eds Costa, E. & Trabucchi, M.) 71–87 (Raven, New York, 1978).
- Lindvall, O. & Björklund, A. *Acta physiol. scand. Suppl.* **412**, 1–48 (1974).
- Kelly, P. H., Seivour, P. W. & Iversen, S. D. *Brain Res.* **94**, 507–522 (1975).
- Nowicky, M. C., Walters, J. R. & Roth, R. H. *J. neural transmission* **42**, 99–116 (1978).
- Phillips, A. G. & Fibiger, H. C. *Can. J. Psychol.* **32**, 58–66 (1978).
- Iversen, S. D. in *Psychobiology of the Striatum* (eds Cools, A. R., Lohman, A. H. M. & van den Bercken, J. H. L.) 99–118 (North-Holland, Amsterdam, 1977).
- Belluzzi, J. D. & Stein, L. *Nature* **266**, 556–558 (1977).

Brain ACTH and endorphin reduced in rats with monosodium glutamate-induced arcuate nuclear lesions

IMMUNOASSAY and immunocytochemical techniques, in some instances with additional characterisation by bioassay and gel filtration, have demonstrated the presence of ACTH-like^{1–10} β -lipotropin (LPH)-like^{6,8,9,11–13} and β -endorphin-like^{6,14,15} activity within the central nervous system. However, the source of these peptides in the central nervous system is uncertain³. Hypophysectomy is not associated with any change in hypothalamic content of ACTH^{1,5,10} or of β -endorphin-like material¹⁴, suggesting a non-pituitary, presumably central nervous system, origin. Immunoreactive perikarya containing

ACTH-like^{8–10,16,17}, β -LPH-like^{8,9,11,13,16,17} and β -endorphin-like^{15–17} material have thus far only been demonstrated in the arcuate nucleus and adjacent lateral areas of the mediobasal hypothalamus. We considered that if the arcuate nucleus were the source of ACTH-like activity within other areas of the central nervous system, its destruction would result in a decrease of such activity. Neonatal administration of sodium glutamate produces central nervous system lesions, restricted largely to neurones of the arcuate nucleus and to the retina¹⁸, while sparing fibres of passage¹⁹. We report here that, using neonatal sodium glutamate administration to produce such arcuate nucleus destruction in rats, the content of ACTH and β -endorphin-like material in the hypothalamus and other brain regions is markedly reduced in the absence of any change in whole pituitary, anterior lobe or plasma ACTH concentrations.

Timed pregnant Holzman rats were housed in air-conditioned quarters on a lighting schedule of 14 h light, 10 h darkness with *ad libitum* food and water. Female offspring received 2.0 mg per g monosodium glutamate (MSG) intraperitoneally on the 2nd and 4th days of life, and 4.0 mg per g on the 6th, 8th and 10th days of life²⁰. Animals were killed at 5 months of age. Another group of animals received five doses of 10% NaCl as an isosmotic control.

Animals treated with MSG demonstrated the endocrinological, metabolic and behavioural abnormalities and the hypothalamic neurotoxicity (arcuate nucleus) characteristic of the MSG syndrome, in confirmation of our previous studies^{20,21}. Animals were decapitated, brains rapidly removed and mediobasal hypothalamus, medial preoptic area, amygdala and sections of cortex removed as previously described²². Five sections of a given area were homogenised in 2 ml 0.1 M HCl, a total of four pools of a given area being obtained from 20 animals. Anterior and neurointermediate pituitary lobes were separately removed, individually placed in 100 μ l HCl and frozen for subsequent assay. At that time 0.9 ml 0.1 M HCl was added to anterior and 0.4 ml to neurointermediate lobe and these were homogenised in a siliconised tube with a Teflon pestle. Trunk blood was obtained at the time of decapitation for immunoassay of plasma ACTH. Determination of immunoreactive ACTH¹ and endorphin²³ content of brain areas and of pituitary lobes was carried out as previously described; an aliquot of the homogenate was removed for measurement of protein concentration²⁴. Immunoassay of plasma ACTH was carried out by methods used in our laboratory²⁵. The ACTH antibody used exhibits approximately 8–30% cross-reactivity with the 31,000 molecular weight (31K) molecule, reacts on an almost equimolar basis with ACTH_{1–24} and ACTH_{1–39}, and exhibits no cross-reactivity with α -MSH, β -MSH, ACTH_{1–10}, ACTH_{25–39}, β -LPH or α -, β -, or γ -endorphin. The β -endorphin antibody reacts with β -LPH, 31K ACTH and β -endorphin on a near-equimolar basis, but does not cross-react with γ -LPH, β -MSH or the enkephalins.

Glutamate-treated animals exhibited a highly significant reduction in immunoreactive concentrations of ACTH-like and β -endorphin-like material in the mediobasal hypothalamus, and also in the medial preoptic nucleus and amygdala. There were no significant changes in whole pituitary, anterior lobe or plasma ACTH content (Table 1). No significant difference in any of these parameters was noted among animals in different oestrous cycle stages.

These findings demonstrate: (1) that destruction of cell bodies of the arcuate nucleus is associated with a four- to fivefold decrease in detectable ACTH-like and endorphin-like immunoreactive material within the hypothalamus, and (2) that this occurs in the absence of any changes in whole pituitary, anterior lobe or plasma ACTH concentrations. These findings indicate that the arcuate nucleus is a major source of the cell bodies giving rise to fibre tracts containing ACTH and endorphin detected in hypothalamic and other sites. The lesser decreases in amygdalar concentrations might indicate that at least some of the immunoreactive material present in this area could arise locally, as suggested by Pacold *et al.*⁵, or that

Table 1 Effect of monosodium glutamate on CNS content of ACTH and β -endorphin

Region	(n)	ACTH (fmol per 100 μ g protein)			β -Endorphin (fmol per 100 μ g protein)		
		Control	MSG	P	Control	MSG	P
Medial basal							
Hypothalamus	4*	52.7 $\pm$ 5.7	9.6 $\pm$ 2.8	<0.001	99.6 $\pm$ 6.6	22.7 $\pm$ 9.8	<0.001
Medial preoptic area	4*	32.7 $\pm$ 6.5	7.9 $\pm$ 3.2	<0.05	117.0 $\pm$ 32.6	35.6 $\pm$ 11.2	<0.05
Amygdala	4*	30.2 $\pm$ 4.8	14.1 $\pm$ 5.4	<0.1	25.1 $\pm$ 2.4	12.4 $\pm$ 1.7	<0.05
Cortex	4*	0.7 $\pm$ 0.2	1.1 $\pm$ 0.1	NS	<0.7	<0.7	
Pituitary (pmol)	20	168.0 $\pm$ 7.3	159.0 $\pm$ 11.5	NS			
Anterior pituitary (pmol)	20	125.5 $\pm$ 7.7	133.1 $\pm$ 11.7	NS			
Plasma (fmol ml ⁻¹)	20	36.0 $\pm$ 5.5	30.9 $\pm$ 6.4	NS			

*Four pools, five animals each (mean $\pm$ s.e.m.).

NS, not significant.

surviving cell bodies (possibly in the mediobasal hypothalamic area adjacent to the arcuate nucleus) preferentially innervate the amygdala. Watson, Richard and Barchas¹⁰ have noted that when the β -LPH-positive cells (arcuate nucleus) were destroyed by hypothalamic lesions on one side of rat brain, there was also a reduction of staining for ACTH and β -LPH fibres.

Additional studies in enucleated animals, animals with stereotaxically produced arcuate nuclear lesions, and deafferentation should show whether other areas affected by MSG administration are involved in the decrease in brain ACTH concentration seen in such treated animals. It is also unlikely that any of the endocrine effects associated with MSG administration²¹ (decreased growth, hypothyroidism and hypogonadism) are responsible, because, as noted, hypophysectomy is not associated with any change in brain ACTH concentration.

The data also demonstrate that brain concentrations of a pituitary-like peptide can vary independently of pituitary or plasma concentrations of that peptide. Similarly, marked alterations in pituitary and plasma ACTH concentrations following short or long term adrenalectomy are not associated with changes in median eminence or mediobasal hypothalamic ACTH concentrations²⁶. This, in addition to the data derived from hypophysectomised animals, suggests a non-pituitary source of these peptides, in keeping with our recent demonstration of synthesis of immunoreactive 31K ACTH/endorphin-like material²⁷ on incubation of dispersed bovine arcuate nucleus area with labelled precursor amino acid. (If ACTH/endorphin-like activity detected in arcuate nucleus perikarya represented a specific uptake and concentrating system of pituitary-derived material, with subsequent axonal transport and processing, this might explain the present findings, but not those of the labelled amino acid incubation studies.)

The MSG-lesioned animal might serve as a model to explore some of the behavioural effects of ACTH-related peptides, although the multiplicity of effects²¹ on other endocrine systems and neurotransmitters would limit the interpretations and specificity of such observations.

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- Krieger, D. T., Liotta, A. & Brownstein, M. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 648–652 (1977).
- Krieger, D. T., Liotta, A. & Brownstein, M. J. *Brain Res.* **128**, 575–579 (1977).
- Moldow, R. & Yalow, R. S. *Proc. natn. Acad. Sci. U.S.A.* **75**, 994–998 (1978).
- Orwoll, E., Gaudette, D. & Kendall, J. *Clin. Res.* **26**, 148A (abstr.) (1978).
- Pacold, S. T., Kirsteins, L., Hojvat, S., Lawrence, A. M. & Hagen, T. C. *Science* **199**, 804–805 (1978).
- Krieger, D. T., Liotta, A., Suda, T., Palkovits, M. & Brownstein, M. J. *Biochem. biophys. Res. Commun.* **76**, 930–936 (1977).
- Guillemin, R., Schally, A. V., Lipscomb, H. S., Andersen, R. N. & Long, J. M. *Endocrinology* **70**, 471–477 (1962).
- Nilaver, G. *et al. J. Cell Biol.* (in the press).
- Pelletier, G., Désy, L., Côté, J., Antakly, T. & Dubé, D. *Endocr. Soc., 60th Ming* (abstr.), 221 (1978).
- Watson, S. J., Richard, C. W. III & Barchas, J. D. *Science* **200**, 1180–1182 (1978).
- Watson, S. J., Barchas, J. D. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5155–5158 (1977).
- Pelletier, G., Désy, L., Lissitzky, J. C., Labrie, F. & Li, C. H. *Life Sci.* **22**, 1799–1804 (1978).
- Zimmerman, E. A., Liotta, A. & Krieger, D. T. *Cell Tissue Res.* **186**, 393–398 (1978).
- Rossier, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 5162–5165 (1977).
- Bloom, F. E. *et al. in The Endorphins* (eds Costa, E. & Trabucchi, M.) 89–109 (Raven, New York, 1978).
- Watson, S. J., Akil, H., Richard, C. W. III & Barchas, J. D. *Nature* **275**, 226–228 (1978).
- Bloch, B., Bugnon, C., Fellman, D. & Lenys, D. *Neurosci. Lett.* **10**, 147–152 (1978).
- Olney, J. W. J. *Neuropath. exp. Neurol.* **33**, 75–90 (1974).
- Paull, W. K. & Lechan, R. *Anat. Rec.* **178**, 436 (abstr.) (1971).
- Nemeroff, C. N. *et al. Endocrinology* **101**, 613–622 (1977).
- Greeley, G. H., Nichol, G. F., Nemeroff, C. B., Youngblood, W. W. & Kizer, J. S. *Endocrinology* **103**, 170–175 (1978).
- Brownstein, M., Arimura, A., Sato, H., Schally, A. V. & Kizer, J. S. *Endocrinology* **96**, 1456–1461 (1975).
- Liotta, A. S., Suda, T. & Krieger, D. T. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2950–2954 (1978).
- Lowry, O. H., Rosenbrough, N. J., Faur, A. L. & Randall, R. S. *J. biol. Chem.* **193**, 265–275 (1951).
- Liotta, A. & Krieger, D. T. *J. clin. Endocr. Metab.* **40**, 268–277 (1975).
- Krieger, D. T., Liotta, A. S., Hauser, H. & Brownstein, M. J. *Endocrinology* (in the press).
- Liotta, A. S., Gildersleeve, D., Brownstein, M. J. & Krieger, D. T. *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Nicotinamide is a brain constituent with benzodiazepine-like actions

BENZODIAZEPINES are a group of drugs with wide therapeutic application as anxiolytics, hypnotics, anticonvulsants and muscle relaxants. To exert their therapeutic and pharmacological effects, they apparently interact with a brain-specific benzodiazepine receptor characterised by a high-affinity binding site for this group of compounds^{1,2}. In analogy to the identification of morphine receptors in the brain and the subsequent isolation of opioid peptides, the discovery of the benzodiazepine receptor prompted the search for an endogenous brain constituent, which physiologically may display benzodiazepine-like actions³. We describe here the isolation and benzodiazepine-like actions of nicotinamide, a compound which might exert these actions in the brain physiologically.

From acetone or HClO₄ extracts of bovine or rat brain, three compounds were isolated by conventional column chromatography and identified by mass spectrometry, which showed some, although rather low, potency in inhibiting specific binding of ³H-diazepam to the benzodiazepine high-affinity binding site, tested *in vitro*¹: nicotinamide (IC₅₀ = 3.9 mM), hypoxanthine

($IC_{50} = 0.7$ mM) and inosine ($IC_{50} = 0.9$ mM). Of these, only nicotineamide showed the main neuropharmacological central effects characteristic of a benzodiazepine, described below, although, because only 0.3% enters the brain after injection into the internal carotid artery⁴, rather high doses of the compound are needed to elicit central effects after systemic application. However, nicotineamide showed the following effects on cat spinal cord activities, recorded as described previously^{5,6}, after local application by pressure ejection from a cannula ($0.5 \mu\text{l}$, 1×10^{-4} M) placed close to the stimulating electrode in the dorsal horn. (1) Dorsal root reflexes (DRRs) induced by dorsal root stimulation were increased by 30–50%. (2) The excitability of primary afferents, as measured by antidromic potentials (APs) in dorsal rootlets, was unaffected, indicating a lack of direct interaction of nicotineamide with γ -aminobutyric acid (GABA) receptors. (3) Unconditioned monosynaptic ventral root reflexes (VRRs) were not affected. In contrast, presynaptic inhibition of monosynaptic VRRs, induced by conditioning and testing stimuli given to appropriate antagonistic muscle nerve afferents in the hind limb, was increased by 15–20% (Fig. 1). The GABA receptor blocker, (+)bicuculline (0.5 mg per kg, intravenously (i.v.)), inhibited these effects of nicotineamide.

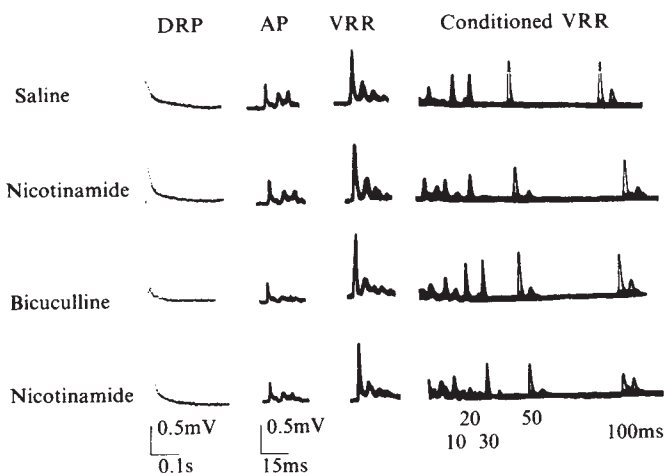


Fig. 1 Enhancement of presynaptic inhibition in cat lumbo-sacral spinal cord by nicotineamide and its reversal by (+)bicuculline. Spinal cord activities of unanaesthetised spinal cats were recorded as described previously^{5,6}. Records are oscilloscope sweeps from one experiment, representative for all five cats tested, showing in four vertical columns from left to right: (1) Averaged dorsal root potentials (DRPs) including the superimposed dorsal root reflexes (eight consecutive responses) evoked in a dorsal rootlet (DR) L_7 by single supramaximal stimuli applied to a DR S_1 ; (2) antidromic potentials (APs) induced in a DR L_7 by constant submaximal volleys given by a semimicroelectrode to the dorsal horn S_1 ; (3) mono- and polysynaptic ventral root reflexes (VRRs) induced in a ventral rootlet S_1 by supramaximal stimuli applied to the nerve afferents of the gastrocnemius muscle; (4) same response as in (3) but now elicited in intervals of approximately 10, 20, 30, 50 and 100 ms following conditioning shocks to the nerve of the biceps femoris muscle. Saline and drugs were administered in the following sequence. Saline: recordings taken 3 min after pressure ejection of saline ($0.5 \mu\text{l}$) through a cannula (outer diameter $100 \mu\text{m}$) in close proximity to the stimulating electrode in the dorsal horn S_1 . Nicotinamide: recordings taken 3 min after the local application of nicotineamide ($0.5 \mu\text{l}$, 1×10^{-4} M); dorsal root reflexes and presynaptic inhibition of conditioned monosynaptic VRRs were augmented. Bicuculline: (+)bicuculline (0.5 mg per kg, i.v.) injected when maximal effects of nicotineamide had been reached; reversed all effects of nicotineamide. Nicotinamide: the action of (+)bicuculline was overcome by the subsequent local injection of nicotineamide ($0.5 \mu\text{l}$, 1×10^{-3} M). Interestingly, in this experiment the presynaptic inhibition by nicotineamide was more pronounced at the 100-ms interval than at shorter intervals, an effect observed only in two out of five cats.

However, the antagonistic action of (+)bicuculline was overcome by the subsequent administration of nicotineamide either locally (1×10^{-3} M, $0.5 \mu\text{l}$) (Fig. 1) or i.v. (300 mg per kg).

Electrophysiological effects, very similar in size and latency to those observed after local application of nicotineamide, were induced by local administration of the benzodiazepine⁷ Ro 11-7800 ($0.5 \mu\text{l}$, 1×10^{-4} M) or an i.v. injection of diazepam (0.1 mg per kg). The action of both drugs was also reversed by (+)bicuculline. Nicotinic acid (1×10^{-3} M, $0.5 \mu\text{l}$ applied locally) had no effect on the cat spinal cord activities tested, indicating that the effect of nicotineamide was not due to a possible conversion to nicotinic acid. Inosine and hypoxanthine applied in the same way and at similar concentrations to nicotineamide were without effect on spinal cord activity.

In a conflict test, nicotineamide restored punishment-suppressed behaviour of rats, an action characteristic of clinically effective anti-anxiety agents. In a 1-h test session, in which level pressing for a food pellet reward was combined with an electric foot shock, pretreatment with nicotineamide increased moderately (500 mg per kg, intraperitoneally (i.p.)) and strongly ($1,000$ mg per kg, i.p.) punished responding in 5 out of 10 animals.

The anticonvulsant action of nicotineamide was established in an assay highly sensitive for the anticonvulsant action of benzodiazepines. 3-Mercaptopropionic acid (MPA), an inhibitor of the GABA synthesising enzyme glutamic acid decarboxylase, was administered in a dose (50 mg per kg i.p.) precipitating seizures in 100% of the mice (20 animals) within 2–3 min. Nicotinamide (500 mg per kg, i.p.), injected 30 min before MPA, protected 25% of the animals from seizures. Hypoxanthine (500 and $1,000$ mg per kg) was without protective effect and inosine was protective (25%) only at $1,000$ mg per kg. Nicotinamide has also been shown to protect against audiogenic^{8,9} and isoniazid-induced¹⁰ seizures.

An anti-aggressive action of nicotineamide was demonstrated by the inhibition of aggressive rage reactions in unrestrained cats elicited by hypothalamic stimulation through chronically implanted electrodes¹¹. The current threshold needed to evoke a hissing response and an attack response was elevated by 15% ($P < 0.01$) after i.p. administration of nicotineamide (500 mg per kg, 4 cats) or diazepam (0.5 mg per kg, 4 cats) but not after inosine (500 mg per kg, 4 cats).

The muscle relaxant action of nicotineamide (300 mg per kg, i.p.) was observed in cats and rats. The sedative effect of the compound was apparent in cats (500 mg per kg, i.p.), confirming previous findings in rats^{12,13}, mice and gerbils^{13,14}.

Most remarkably, nicotineamide shows hypnotic action, previously analysed in mice and in man (250 mg per kg subcutaneously and 3 g d^{-1} , orally respectively). It manifests itself as a prolongation of total sleep time and an increase of rapid eye movement sleep^{15–17}, a response also seen after low doses of benzodiazepines^{18,19}. Finally, the finding that nicotineamide treatment increases the 5-hydroxytryptamine level in rat brain¹² is in line with the suggestion that the anxiety-reducing action of benzodiazepines is mediated by the reduction of 5-hydroxytryptamine turnover^{20,21}.

Thus, nicotineamide has properties in common with benzodiazepines (and barbiturates²²) in its action on spinal cord activity, its anticonflict, anticonvulsant, anti-aggressive, muscle relaxant and hypnotic action. It also seems to influence GABAergic mechanisms without binding to the GABA receptor itself, as shown by the electrophysiological findings and the lack of inhibition by nicotineamide of ^3H -GABA receptor binding tested according to Enna *et al.*²³.

If nicotineamide physiologically exerts benzodiazepine-like effects in the brain, its pharmacological potency should be high in view of its low concentration in the brain ($0.1 \mu\text{mol}$ per g rat whole brain)²⁴. This is supported by the electrophysiological experiments using *in situ* drug application, in which the potency of nicotineamide was found to be equivalent to that of a highly potent benzodiazepine. The low apparent affinity of nicotineamide to the benzodiazepine binding site *in vitro* may either

be due to shortcomings of the binding assay in reflecting the *in vivo* affinity of the endogenous ligand to that site, or the benzodiazepine receptor may be part of a receptor complex²⁵, which may contain more than one binding site through which benzodiazepine-like actions can be elicited in the brain. The hypothesis that nicotinamide may exert benzodiazepine-like actions in the brain physiologically, may shed new light on the mental disorders accompanying nicotinamide deficiency states.

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1. Möhler, H. & Okada, T. *Science* **198**, 849–851 (1977).
2. Squires, R. F. & Braestrup, C. *Nature* **266**, 732–734 (1977).
3. Iversen, L. *Nature* **275**, 477 (1978).
4. Deguchi, T., Ichijima, A., Nishizuka, Y. & Hayaishi, O. *Biochim. biophys. Acta* **158**, 382–393 (1968).
5. Polc, P., Möhler, H. & Haefely, W. *Archs Pharmac.* **284**, 319–337 (1974).
6. Polc, P. & Haefely, W. *Archs Pharmac.* **300**, 199–203 (1977).
7. Curtis, D. R., Lodge, D., Johnston, G. A. R. & Brand, S. J. *Brain Res.* **198**, 344–347 (1976).
8. Maitre, M., Ciesielski, L., Lehmann, A., Kempf, E. & Mandel, P. *Biochem. Pharmac.* **23**, 2807–2816 (1974).
9. Lehman, A., Simler, S. & Mandel, P. *J. Physiol. (Paris)* **55**, 446 (1977).
10. Balzer, H., Holtz, P. & Palm, D. *Naunyn-Schmiedeberg's, Archs Pharmac.* **239**, 520–552 (1960).
11. Brown, J. L., Hunsperger, R. W. & Rosvold, H. E. *Expl Brain Res.* **8**, 113–129 (1969).
12. Scherer, B. & Drämer, W. *Life Sci.* **11**, 189–195 (1972).
13. Wooley, D. W. *Science* **128**, 1277–1278 (1958).
14. Beaton, J. M. *Experientia* **32**, 1036–1037 (1976).
15. Beaton, J. M., Pegram, G. V., Smythies, J. R. & Bradley, R. J. *Experientia* **30**, 926–927 (1974).
16. Pegram, G. V., Robinson, C., Donaldson, P., Beaton, J. & Smythies, J. *2nd Int. Sleep Res. Cong.*, Edinburgh, 91 (1975).
17. Robinson, C. R., Pegram, G. V., Hyde, P. R., Beaton, J. M. & Smythies, J. *Biol. Psychiatry* **12**, 139–143 (1977).
18. Polc, P. & Haefely, W. in *Sleep* (eds Levin, P. & Koella, W. P.) 303–305 (Karger, Basle, 1974).
19. Tissot, R. *Prog. Brain Res.* **18**, 175–177 (1965).
20. Stein, L., Wise, C. D. & Belluzzi, J. D. *Adv. biochem. Psychopharmac.* **14**, 29–44 (1975).
21. File, S. E. & Velucci, S. V. *J. Pharm. Pharmac.* **30**, 105–110 (1978).
22. Haefely, W. *Ag. Actions* **7**, 353–359 (1977).
23. Enna, S. J. & Snyder, S. H. *Brain Res.* **100**, 81–97 (1975).
24. Gerber, G. B. & Deroo, J. *Proc. Soc. exp. Biol. Med.* **134**, 689–693 (1970).
25. Guidotti, A., Toffano, G. & Costa, E. *Nature* **275**, 553–555 (1978).

Immunocytochemical localisation of L-glutamic acid decarboxylase in the goldfish retina

A DIRECT way to examine whether certain neurones may use γ -aminobutyric acid (GABA) as a neurotransmitter is to determine by immunocytochemistry if L-glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, is localised in these cells. We have been studying GABA-ergic pathways in the goldfish retina by autoradiographic and biochemical methods^{1–3}. It would therefore be of interest to localise the GAD-containing cells in this system immunocytochemically. However, although GAD has been purified from mouse brain⁴ and antibodies against the enzyme have been obtained, these antibodies do not cross-react with the GAD found in neural tissues of teleosts⁵. Thus, before immunocytochemical studies of GAD-containing neurones in the goldfish retina were possible, we had to purify GAD from teleost brain and obtain a specific antibody against this enzyme. Such an antibody has now been obtained⁶. We show here that in the goldfish retina, GAD is localised in specific interneurons: type H1 horizontal cells and probably type Ab pyriform amacrine cells. These cell types

have been shown to be both pre- and postsynaptic to red-sensitive cones and red-sensitive centre-depolarising bipolar cells, respectively^{3,7–9}.

Because we were able to obtain the channel catfish (*Ictalurus punctatus*) in large numbers, catfish brains were used for the purification of GAD⁶. The enzyme was extracted by homogenising 600 brains (about 300 g) in 3 l of water containing 0.1 mM pyridoxal phosphate, 1 mM EDTA, 1 mM 2-aminoethylisothionium bromide hydrobromide and 1 mM reduced glutathione, pH 7.2. About 70% of the GAD activity as measured by formation of both ¹⁴C-CO₂ and ¹⁴C-GABA (refs 4, 10) which was recovered in the supernatant after centrifugation of the homogenate at 100,000g for 45 min. The enzyme was purified to electrophoretic homogeneity by a combination of ammonium sulphate fractionation, gel filtration, calcium phosphate gel and preparative gel electrophoresis. Doses of 20 μ g of this enzyme were injected biweekly into two rabbits for 2 months. The ear veins were bled and serum was prepared by centrifugation at 2,000g for 20 min and used for immunocytochemical studies. Anti-catfish GAD immunoglobulins (IgG) from rabbit were prepared by passing the serum through a DEAE Sephacel ion-exchange column and eluting with 50 mM Tris buffer, pH 8.0. The IgG were used for immunodiffusion studies⁶.

GAD-containing neurones in the goldfish retina were localised immunocytochemically using the method of Dubois-Dalcq *et al.*^{11,12}. Eyes from light-adapted goldfish (*Carassius auratus*) were enucleated and isolated eyecups were fixed with 4% paraformaldehyde in isotonic phosphate-buffered saline (PBS; 100 mM NaCl and 25 mM sodium phosphate, pH 7.2) for 1 h at room temperature and overnight at 4 °C. Each eyecup was then washed with PBS, cut radially into strips several mm wide, embedded in agar and sectioned at 50 μ m with a vibratome. Sections were incubated at room temperature for 2 h with a 1:250 dilution of rabbit-anti-catfish GAD serum (1:50 to 1:1,000 dilutions of the antiserum give similar staining patterns) or non-immunised serum (control), then washed with PBS and

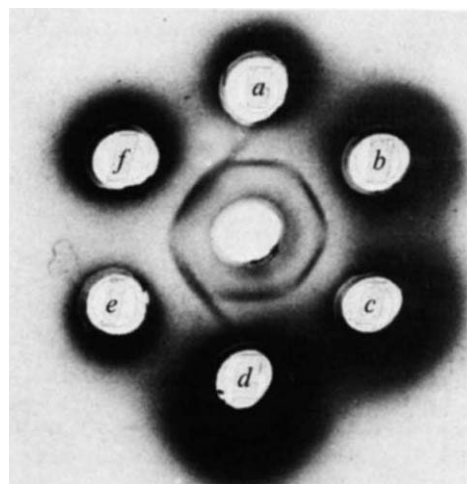


Fig. 1 Ouchterlony double diffusion test with crude GAD from goldfish brain, goldfish retina and catfish brain. Each well contained 15 μ l of solution. Anti-catfish GAD IgG (70 μ g) was in the central well. Wells a to f were homogenates from goldfish brain (a, b), goldfish retina (c, d) and catfish brain (e, f), respectively. The diffusion plate was kept at 4 °C for 48 h, washed with PBS at room temperature for 48 h, stained with 1% Coomassie blue for 20 min and destained with 7% acetic acid for 2 h. The spurs between f and a, and d and e suggest that catfish and goldfish GAD are not identical enzymes. However, the formation of a single, sharp precipitin band in b and c indicates that the antibody against catfish GAD cross-reacts strongly and specifically with goldfish GAD.

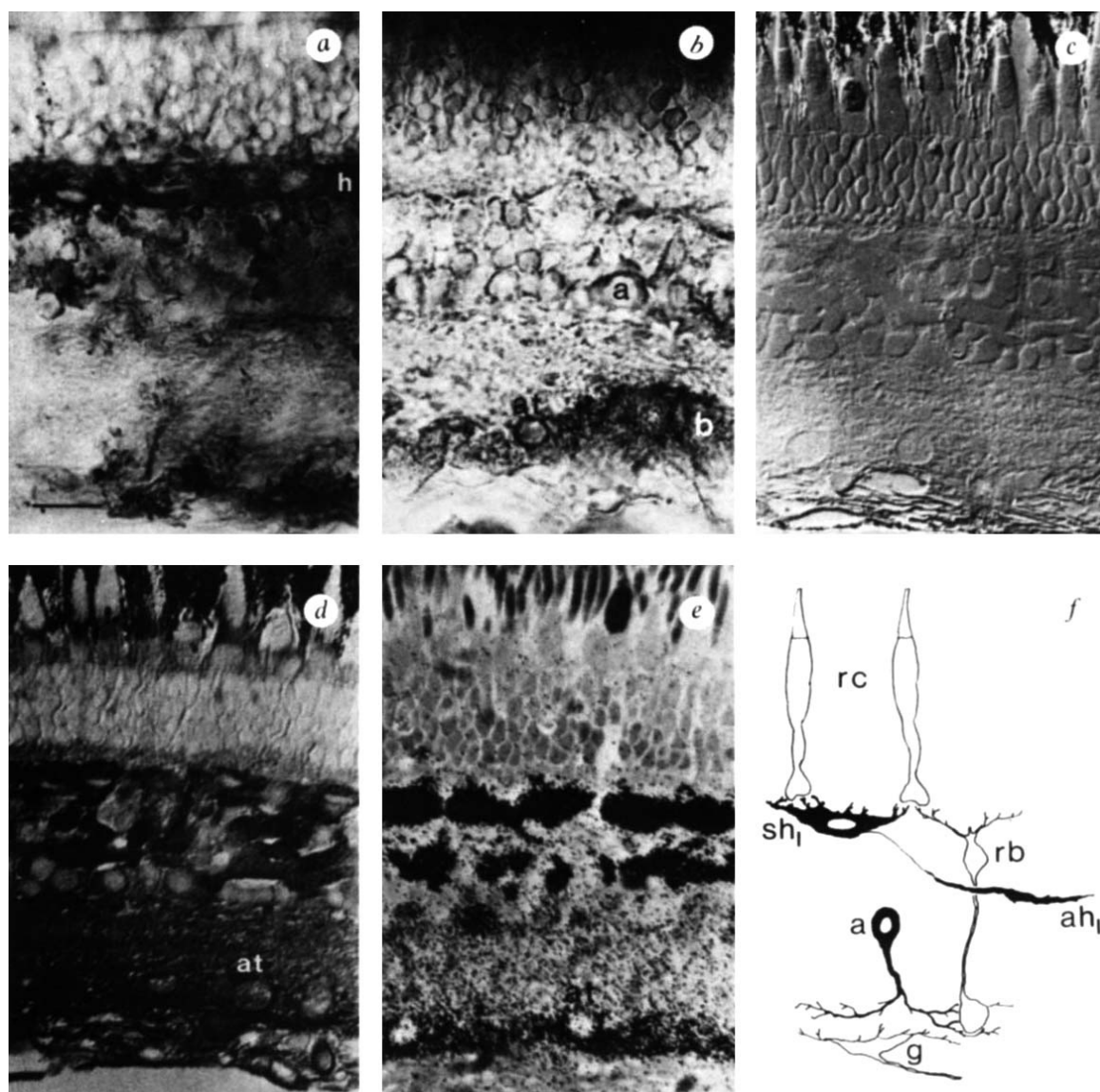


Fig. 2 *a, b*, Immunocytochemical localisation of GAD in 50-μm sections of goldfish retina. Focusing on the outer retina (*a*), HRP-staining is seen over the somata of some cone horizontal cells (*h*). Note that the nuclei of these cells are not stained and therefore probably do not contain GAD. Scale bar 20 μm. Focusing on the inner retina (*b*), HRP-staining is seen over some amacrine cells (*a*, the nucleus is not stained) and in sublamina b of the inner plexiform layer, indicating that GABAergic terminals are probably concentrated in this sublamina (*b*). Note dense HRP-staining around the axon terminal (*at*) of a type b bipolar cell^{3,8,9}. *c*, Control experiment, where a section treated with non-immunised serum shows very little HRP staining. The dark structures between photoreceptors are melanin granules in pigment epithelial cells. *d*, HRP-staining of GAD in a 5-μm section shows that some somata and axons of cone horizontal cells, probably H1 cells are stained. Sublamina b of the inner plexiform layer is also stained, especially around the axon terminal of some type b bipolar cells. *e*, Autoradiographic section of a goldfish retina incubated with 10^{-6} M 3 H-GABA. As shown earlier^{1,3}, high-affinity GABA uptake is localised in the somata and axons of H1 horizontal cells, and in some Ab pyriform amacrine cells which make synaptic connections in sublamina b of the inner plexiform layer. *f*, Schematic diagram of probable GABA-ergic neurons (H1 horizontal cells and some Ab amacrine cells) and their functional connections in the goldfish retina as revealed by our immunocytochemical and autoradiographic studies. rc: red-sensitive cones; sh₁ and ah₁: soma and axon of a H1 cell; rb: red-sensitive centre-depolarising bipolar cell (type b); a: pyriform amacrine cell which makes contact with rb; g: retinal ganglion cell.

treated for 2 h with a conjugate of horseradish peroxidase (HRP) and protein A¹¹. After washing, the sections were incubated with diaminobenzidine and hydrogen peroxide¹³. The sections were then fixed in 3% glutaraldehyde in 100 mM phosphate buffer (pH 7.4) for 1 h, dehydrated in ethanol, rinsed with xylene, and immersed in cedarwood oil. The HRP reaction products in these sections were visualised by Nomarski differential interference microscopy. The sections were then embedded in a thin layer of epoxy and sectioned tangentially at 2–5 μm for light microscopy.

Several lines of evidence indicate that the GAD purified from catfish brain and used as the antigen is homogeneous, and that

the antibody against catfish GAD is monospecific⁶. First, in several different polyacrylamide gel electrophoresis systems, the purified enzyme migrated as a single band which contained all the GAD activity. Second, immunoprecipitation studies showed that the antibody inhibited 67% of the GAD activity from catfish brain. Third, the Ouchterlony double immunodiffusion test showed only one sharp precipitin band between the antibody and catfish brain extract (Fig. 1). Finally, this precipitin band contained 86% of the GAD activity on the immunodiffusion gel⁶.

The formation of only one sharp precipitin band between the anti-GAD serum and goldfish brain and retina shows that

goldfish GAD cross-reacts strongly with this antibody. Using the antibody, our immunocytochemical study shows that GAD is localised in some horizontal cells, a few amacrine cells and sublamina b of the inner plexiform layer (Fig. 2). In the goldfish retina, different types of horizontal cells can be classified physiologically and morphologically according to their functional contacts with photoreceptors⁷. Thus, rod horizontal cells receive input exclusively from rods, and cone horizontal cells can be subdivided into H1, H2 and H3 cells, which receive input predominantly from red, green and blue-sensitive cones, respectively. The positions of the HRP-labelled horizontal cells in the inner nuclear layer indicate that these are the perikarya of H1 horizontal cells. To enhance HRP staining in cell bodies and axons of GAD-containing cells, 50- μ m sections were incubated with 0.1% Triton X-100 and the anti-GAD serum (a 1:4,000 dilution was used for most Triton X-100 treated tissue) overnight before the normal immunocytochemical procedure¹⁴. With this treatment, both the perikarya and axons of some horizontal cells are heavily stained (Fig. 2). Again, the positions of the HRP-stained cell bodies indicate that these are H1 horizontal cells. It follows that the HRP-stained axons are most probably axons of H1 cells. H2, H3 and rod horizontal cells, photoreceptors, bipolar cells and Müller (glial) cells do not show detectable HRP staining for this enzyme.

The identities of the HRP-stained amacrine cells cannot be determined without serial reconstruction and electron microscopic studies. The presence of GAD in sublamina b of the inner plexiform layer, especially around the axon terminals of some centre-depolarising bipolar cells (type b)^{8,9}, suggests that GAD-containing amacrine cells make synaptic contacts with these bipolar cells. This result and those of autoradiographic studies of ³H-GABA uptake in the inner plexiform layer, indicate that at least some GABA-ergic amacrine cells are Ab pyriform cells which make contact with red-sensitive centre-depolarising bipolar cells in the goldfish retina^{3,8,9}.

Comparison between the immunocytochemical studies of GAD-containing neurones and autoradiographic studies of GABA uptake reveals a marked similarity in the labelling patterns (Figs. 2, 3). Our findings therefore demonstrate that in goldfish retina, the neurones which possess a high-affinity system for GABA uptake also contain significant levels of GAD. In addition, this study confirms earlier biochemical results showing that at least some isolated axons of cone horizontal cells in the goldfish retina synthesise GABA and contain high specific activities of GAD². These findings, and

those of autoradiographic^{1,2} and electrophysiological¹⁵ studies of the GABA system in the teleost retina, point to GABA as being the transmitter used by H1 horizontal cells of the goldfish retina. The absence of GABA uptake and GAD in all other horizontal cell types (H2, H3 and rod) indicates that they are not GABA-ergic. This raises the possibility that horizontal cells which are in contact with different types of photoreceptors may use different neurotransmitters.

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1. Lam, D. M. K. & Steinman, L. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2777-2781 (1971).
2. Lam, D. M. K. *Nature* **254**, 345-347 (1975); *Cold Spring Harb. Symp. Quant. Biol.* **40**, 571-579 (1975).
3. Marc, R. E., Stell, W. K., Bok, D. & Lam, D. M. K. *J. comp. Neurol.* **182**, 221-246 (1978).
4. Wu, J.-Y. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. N. Tower, D. B.) 7-55 (Raven, New York, 1976).
5. Saito, K., Wu, J.-Y. & Roberts, E. *Brain Res.* **65**, 277-285 (1974).
6. Su, Y. Y. T., Wu, J.-Y. & Lam, D. M. K. *Abstr. Soc. Neurosci. U.S.A.* (1978); *J. Neurochem.* (in the press).
7. Stell, W. K. & Lightfoot, D. O. *J. comp. Neurol.* **159**, 473-502 (1975).
8. Famiglietti, E. V., Kaneko, A. & Tachibana, M. *Science* **198**, 1267-1269 (1977).
9. Stell, W. K., Ishida, A. T. & Lightfoot, D. O. *Science* **198**, 1269-1271 (1977).
10. Lam, D. M. K. *J. Cell Biol.* **54**, 225-231 (1972).
11. Dubois-Dalcq, M., McFarland, H. & McFarlin, D. J. *Histochem. Cytochem.* **25**, 1201-1206 (1977).
12. Brandon, C., Wu, J.-Y. & Lam, D. M. K. *Abstr. Soc. Neurosci. U.S.A.* (1978).
13. Saito, K., Barber, R., Wu, J.-Y., Matsuda, T., Roberts, E. & Vaughn, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 269-273 (1974).
14. Grzanna, R., Molliver M. E. & Coyle, J. T. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2502-2506 (1978).
15. Lam, D. M. K., Lasater, E. M. & Naka, K.-I. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6310-6313 (1978).



Fig. 3 A 3- μ m section of a goldfish retina treated with 0.1% Triton X-100 and anti-GAD serum (1:4,000 dilution) overnight and processed for immunocytochemistry. Because this treatment enhanced the penetration of the antiserum and conjugate into the tissue, HRP staining is much more intense than that shown in Fig. 2. In particular, both the somata and axons of the H1 horizontal cells are clearly stained. Scale bar 20 μ m.

Identification of β -adrenergic-sensitive adenylate cyclase in intracranial blood vessels

THE regulation of cerebral blood flow and vascular permeability is important in such clinical conditions as stroke, arterial spasm, vascular headache (for example, migraine) and metabolic effects of seizures. Unfortunately, relatively little is known about the nervous control of cerebral circulation, particularly with regard to the nature of the hormone and neurotransmitter receptors present in cerebral vessels. An influence of the adrenergic nervous system has been suggested by histological studies showing that the blood vessels and branches of the circle of Willis receive innervation from the sympathetic chain¹, and that the smaller, penetrating cerebral vessels (called microvessels) are surrounded by noradrenaline-containing fibres which seem to arise from brainstem noradrenergic nuclei^{2,3}. Although attempts to demonstrate β -adrenergic receptors in cerebral vascular tissue by cytochemical methods have been unsuccessful⁴, indirect evidence for the presence of such receptors is

suggested by reports that adrenergic stimulation can alter cerebral blood flow and vascular permeability^{3,5}, and that catecholamine agonists (such as isoprenaline and adrenaline) can dilate whole-artery preparations of cerebral vessels⁶. We now report the presence of a β -adrenergic-stimulated adenylate cyclase in both superficial and deep cerebral blood vessels. In addition to supplying direct biochemical evidence for the existence of β -adrenergic receptors in the cerebral vasculature, identification of this enzyme should facilitate the evaluation of the actions of pharmacological agents on cerebral blood flow and vascular permeability.

Previous studies have demonstrated that the receptors for several neurotransmitters and circulating hormones are intimately associated with the membrane-bound enzyme, adenylate cyclase, and that activation of this enzyme, resulting in the intracellular formation of cyclic AMP, mediates the effects of the hormone or neurotransmitter⁷. To demonstrate the possible existence of a β -adrenergic-stimulated adenylate cyclase in cerebral vascular tissue, the effects of various catecholamines on the enzymatic synthesis of cyclic AMP from ATP were studied. Broken cell preparations of three types of mammalian blood vessels were used: superficial arteries and proximal pial branches of the circle of Willis; penetrating (intraparenchymal) microvessels; and extracranial carotid arteries. The microvessels were prepared by a modification of published techniques^{8,9}, involving mechanical fragmentation, discontinuous sucrose gradient centrifugation, and sieve filtration (see Fig. 1 legend). Feline blood vessels were used for most experiments, as previous studies have demonstrated a rich

noradrenergic innervation in the cerebral vasculature of this species^{10,11}, and physiological studies have documented β -adrenergic-mediated vasomotor responses of cat pial arteries⁶. In preliminary experiments, dog and calf pial vessels also demonstrated β -adrenergic-stimulated adenylate cyclase activity, but the degree of stimulation was less than that in the cat.

Figure 1a shows the effects of (-)isoprenaline (Iso), a β -adrenergic agonist, on adenylate cyclase activity in an homogenate of cat pial vessels. In the experiment shown, maximal stimulation by Iso was 160% of control activity and, in other experiments, varied between 150% and 180% of control. Concentrations of Iso as low as 10^{-7} M caused significant stimulation, and half-maximal activity occurred at a concentration (K_a) which varied between 4 and 11×10^{-7} M. (-)Noradrenaline (NA), a mixed β - and α -adrenergic agonist, was less effective than Iso in stimulating enzyme activity (150% of control in pial vessels), whereas (-)phenylephrine, an α -adrenergic agonist, caused only minimal stimulation at comparable concentrations.

Activation of adenylate cyclase activity by Iso and NA was more pronounced in preparations of cat intraparenchymal microvessels (Fig. 1b), varying between 170 and 225% of control activity for Iso and 165 and 220% for NA, at maximum. In the experiment shown, the K_a for Iso was 3×10^{-7} M. In contrast, none of the catecholamines had any effect on the adenylate cyclase activity present in homogenates of cat common and proximal external carotid artery (Fig. 2).

Results similar to those above were obtained with both pial vessels and microvessels from cats which had been perfused systemically to remove intravascular blood. The finding that whole blood, itself, showed little Iso-stimulated enzyme activity, made it unlikely that blood elements contributed to our results. In contrast to the effects observed in vessels, stimulation of whole brain adenylate cyclase by a maximally effective concentration (100 μ M) of Iso was only 128% of control activity (Fig. 2). On the other hand, 100 μ M dopamine (DA) activated the enzyme activity of brain (150% of control) more than that of either pial vessels (130%) or microvessels (124%). Also, further evidence differentiating brain from vessel adenylate cyclase was obtained from the observed effects of GTP, which (at 100 μ M) activated basal enzyme activity of cat brain homogenates by 600% but those of pial vessels and microvessels by only 200%

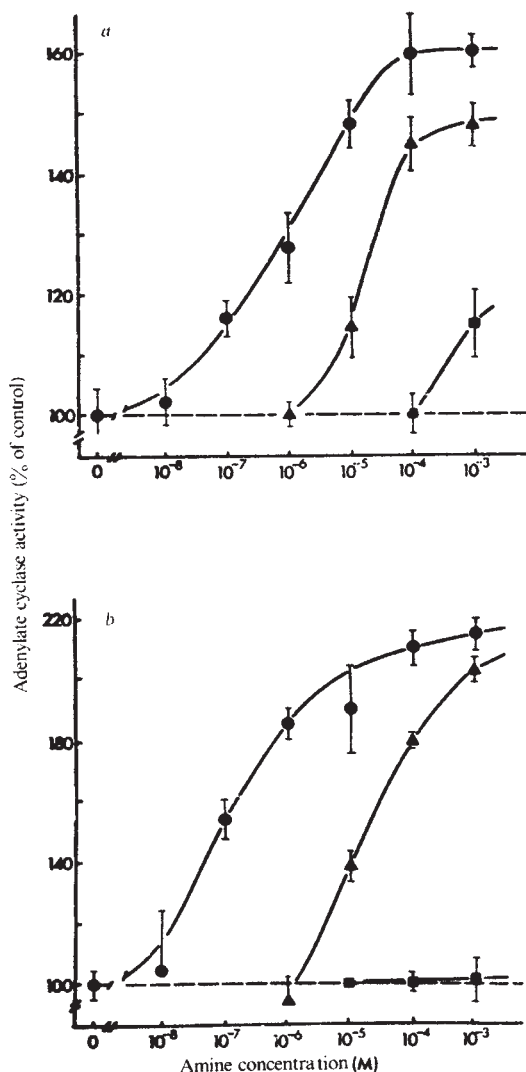


Fig. 1 Effects of (-)isoprenaline (●), (-)noradrenaline (▲) and (-)phenylephrine (■) on adenylate cyclase activity in broken cell preparations of cat cerebral vasculature. *a*, Pial arteries, control activity = 13.8 ± 1.6 pmol per mg protein per min; *b*, intraparenchymal microvessels, control = 11.5 ± 0.7 pmol per mg protein per min. Values shown are the means ($\pm$ deviation) of replicate samples, each assayed for cyclic AMP in triplicate. For these studies, male cats were anaesthetised with ether and perfused transcardially with 500 ml of phosphate-buffered saline (PBS). Superficial arteries (distal vertebral, basilar, intracranial carotid, proximal portions of anterior, middle and posterior cerebral) were dissected and cleaned of adhering pia, rinsed in PBS, and homogenised (20 mg ml⁻¹) in cold 6 mM Tris-maleate buffer, pH 7.4. To isolate microvessels, the brain was stripped of remaining pia, coarsely minced, and gently homogenised by hand (six vertical strokes, no rotation) in a Teflon-glass homogeniser (0.2 mm clearance) in 60 ml cold 25 mM Tris-maleate buffer, pH 7.4, containing 140 mM NaCl and 0.5% bovine serum albumin (buffer A). After centrifugation at 1,500 g for 15 min, the precipitate was resuspended in 2 vols 0.25 M sucrose and centrifuged (25,000 r.p.m. for 50 min) on a discontinuous 1.0/1.8/2.25 M sucrose gradient. The resulting 1.0/1.8 M and 1.8/2.25 M fractions were diluted in buffer A, centrifuged at 4,000 g, the pellet resuspended in 0.25 M sucrose, and centrifuged (25,000 r.p.m. for 40 min) on a 1.0/1.35/1.5/1.7/2.25 M sucrose gradient. The microvessel fraction (combined 1.5/1.7 M and 1.7/2.25 M interfaces) was washed and collected against a 100- μ m Nitex screen and homogenised (15 mg ml⁻¹) in 6 mM Tris-maleate, pH 7.4. Vessel purity was monitored through the use of light microscopy and enzyme markers¹⁵. Cyclic AMP formation was measured in tubes containing (in 0.3 ml) 80 mM Tris-maleate, pH 7.4, 10 mM theophylline, 6 mM MgSO₄, 0.1 mM GTP, 1.5 mM ATP, and tissue homogenate (1.0–1.5 mg wet weight), plus test substances as indicated. The reaction (4 min at 30°C) was initiated by addition of ATP, stopped by heating to 90°C for 2 min, and then centrifuged to remove insoluble matter. Cyclic AMP was measured as in ref. 16. In these conditions, enzyme activity was linear with time and enzyme concentration, and phosphodiesterase activity was nearly completely inhibited. All experiments were replicated 2–5 times. The results shown in Figs 1 and 2 and Table 1 represent typical experiments.

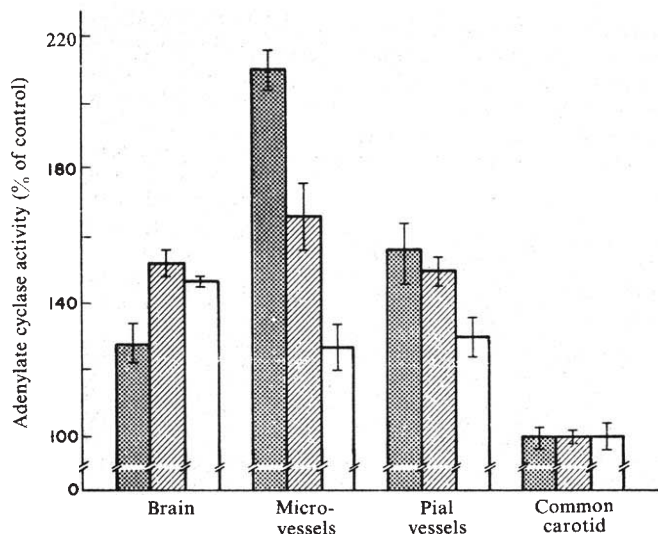


Fig. 2 Comparative effects of 100 μ M (—)isoprenaline (▨), (—)noradrenaline (■) and dopamine (□) on adenylate cyclase activity in homogenates of whole cat brain (control activity = 37 ± 1 pmol per mg protein per min) and three preparations of cat vasculature: intraparenchymal microvessels (control = 13.8 ± 0.6 pmol per mg protein per min); superficial pial vessels (control = 12.3 ± 0.9 pmol per mg protein per min); and common and external carotid artery (control = 1.3 ± 0.2 pmol per mg protein per min). The values shown here and in Table 1 are the means ($\pm$ deviation) of replicate samples, each assayed for cyclic AMP content in duplicate.

and 120%, respectively. In contrast to effects on basal activity, Iso stimulation, itself, had an absolute requirement for the presence of exogenous GTP. Other experiments indicated that, unlike adenylate cyclases activated by DA, histamine and serotonin, present in other tissues⁷, Iso-stimulated adenylate cyclase activity in cerebral vessel homogenates was unaffected by calcium chelation with EGTA (calcium did affect basal activity).

The preferential activation of a β -adrenergic receptor by Iso in cerebral vessel homogenates was supported by other experiments using receptor antagonists. Table 1 shows that Iso-stimulated adenylate cyclase activity in a microvessel homogenate was almost completely blocked by the β -adrenergic antagonist, propranolol, but was less affected by the α -adrenergic

antagonist, phentolamine, or the potent DA antagonist, fluphenazine. Similar results were seen in homogenates of pial vessels. Table 1 also demonstrates the non-additive effects of Iso, NA and DA on microvessel adenylate cyclase, results supporting the activation of a single class of receptors.

Taken together, these data demonstrate the presence of a β -adrenergic-sensitive adenylate cyclase in cat intraparenchymal and extraparenchymal cerebral blood vessels. Because of the known association of adenylate cyclase with β -adrenergic receptors in other tissues^{12,13}, identification of this enzyme strongly supports the existence of a β -adrenergic receptor in the mammalian cerebral vasculature. It will be of interest to determine the cellular localisation of β -adrenergic-sensitive adenylate cyclase within the blood vessel wall; a presence in endothelial cells might suggest an involvement in vascular permeability, or a media localisation, an involvement in vasomotor responses. In addition, further characterisation of the cerebral vasculature β -adrenergic receptor, itself, should prove helpful in evaluating and comparing the actions of adrenergic agents (such as β_1 and β_2 selective types) in both intraparenchymal and extraparenchymal cerebral vessels.

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- Edvinsson, L. *Acta physiol. scand.* **427**, Suppl., 1–35 (1975).
- Hartman, B. K., Zide, D. & Udenfriend, S. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2722–2726 (1972).
- Raichle, M. E., Hartman, B. K., Eichling, J. O. & Sharpe, L. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3726–3730 (1975).
- Melamed, E., Atlas, D. & Lahav, M. *Stroke* **8**, 261–263 (1977).
- Kobayashi, S., Waltz, A. G. & Rhoton, A. L. *Neurology* **21**, 297–302 (1971).
- Edvinsson, L. & Owman, C. *Circulation Res.* **35**, 835–849 (1974).
- Nathanson, J. A. *Physiol. Rev.* **57**, 157–256 (1977).
- Mrsulja, B. B., Mrsulja, B. J., Fujimoto, T., Klatzo, I. & Spatz, M. *Brain Res.* **110**, 361–365 (1976).
- Schivonchick, D. P. & Roots, B. I. *Lipids* **12**, 165–169 (1976).
- Nielsen, K. C. & Owman, C. *Brain Res.* **6**, 773–776 (1967).
- Cervos-Navarro, J. & Matakas, F. *Neurology* **24**, 282–286 (1974).
- Schramm, N. & Naim, E. J. *biol. Chem.* **245**, 3225–3231 (1970).
- Aurbach, G. D., Spiegel, A. M. & Gardner, J. D. *Adv. Cyclic Nucleotide Res.* **5**, 117–132 (1975).
- Nathanson, J. & Glaser, G. *Ann. Neurol.* **4**, 81 (1978).
- Djuricic, B. M. & Mrsulja, B. B. *Brain Res.* **138**, 561–564 (1977).
- Brown, B. L., Elkins, R. P. & Albano, J. D. *Adv. Cyclic Nucleotide Res.* **2**, 25–40 (1970).

Table 1 Effects of various compounds on adenylate cyclase activity

Agent	Cyclic AMP increase (pmol per mg protein per min)
a Antagonists	
Iso (100 μ M)	11.5 $\pm$ 0.9
Iso (100 μ M) + Prop (10 μ M)	4.6 $\pm$ 1.0
Iso (100 μ M) + Phent (10 μ M)	11.1 $\pm$ 0.6
Iso (100 μ M) + Flu (10 μ M)	11.8 $\pm$ 0.4
Iso (100 μ M) + Prop (50 μ M)	1.0 $\pm$ 0.6
Iso (100 μ M) + Phent (50 μ M)	7.3 $\pm$ 0.3
Iso (100 μ M) + Flu (50 μ M)	7.4 $\pm$ 0.4
b Additivity	
Iso (1 mM)	13.1 $\pm$ 0.1
NA (1 mM)	12.0 $\pm$ 0.5
DA (1 mM)	10.4 $\pm$ 0.4
Iso (1 mM) + NA (1 mM)	12.9 $\pm$ 0.4
Iso (1 mM) + DA (1 mM)	12.9 $\pm$ 0.9

a, Effects of the β -adrenergic antagonist, propranolol (Prop), α -adrenergic antagonist, phentolamine (Phent), and dopamine antagonist, fluphenazine (Flu), on activation of adenylate cyclase activity by (—)isoprenaline (Iso) in an homogenate of cat cerebral microvessels. **b**, Effects of maximally effective concentrations of Iso, (—)noradrenaline (NA), and dopamine (DA), alone and in combination, on adenylate cyclase activity in another preparation of microvessels. Qualitatively similar results were obtained in additivity experiments using 10-fold lower amine concentrations. The values shown in **a** are the increases due to Iso over enzyme activity in the presence of antagonist alone. In the absence of added agents, control activity was 13.1 ± 1.1 pmol per mg protein per min in **a** and 17.7 ± 1.1 pmol per mg protein per min in **b**.

Distribution of muscarinic acetylcholine receptors in amphibian cardiac muscle

THE skeletal muscle–nerve junction and many interneuronal synapses are highly specialised and morphologically characterised by a direct apposition of pre- and postsynaptic elements separated by a cleft of no more than 50 nm, localised thickenings of the apposed membranes and an aggregation of small vesicles in the presynaptic process. At several such ‘focal’ synapses, neurotransmitter receptor molecules are restricted to patches of postsynaptic membrane corresponding to synaptic sites^{1–3}. For example, at the motor endplate of skeletal muscle, acetylcholine receptor packing density in the subsynaptic membrane is about 10^3 times higher than in the extrasynaptic membrane several μ m away^{4–6}. Many neuroeffector junctions such as those in autonomically innervated tissues, however, are much less specialised and lack synaptic membrane apposition and thickenings^{7,8}. In the heart for example, the terminal postganglionic autonomic axons consist of long chains of vesicle-packed varicosities and

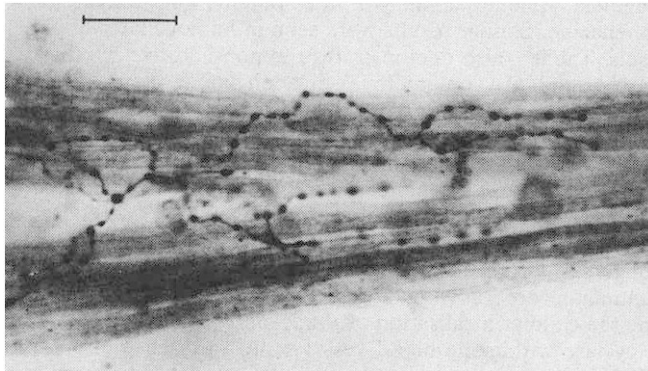


Fig. 1 Two cardiac muscle fibres in a *Xenopus* interatrial septum stained with zinc iodide and osmium. A fresh interatrial septum pinned to a Sylgard-coated cover slip was incubated overnight in ice-cold zinc iodide and osmium. Zinc iodide was made by mixing 2 g Zn dust and 1 g I_2 in 40 ml H_2O . 2 ml of the filtered zinc iodide, 0.5 ml of 2% OsO_4 , and 6 ml of 0.2 M sodium acetate buffer pH 3.9 were mixed to make the zinc iodide and osmium stain (M. Letinsky, personal communication). Scale bar, 20 μm .

form a widely ramifying plexus over the muscle⁹. The varicosities sometimes lie in close apposition to the muscle but may often be several μm away from the nearest target. Even when pre- and postsynaptic membranes are closely apposed, morphological specialisation of the membranes is not normally seen^{7,10-12}. I was interested to determine whether receptor molecules for acetylcholine (ACh) in heart muscle are localised to the relatively infrequent sites of close apposition between nerve and muscle or are more generally distributed. This report demonstrates that muscarinic ACh receptors are uniformly distributed in cardiac muscle fibres in amphibian heart.

Distribution of muscarinic ACh receptors was determined by autoradiography of cardiac muscle labelled with 3H -quinuclidinyl benzylate (QNB). QNB (Roche) has been shown to bind strongly and with high specificity to muscarinic ACh receptors in mammalian cardiac muscle¹³ and other tissues¹⁴. Similar binding properties of QNB in amphibian heart have recently been found (H.C.H., in preparation). Before autoradiography, conditions for labelling muscarinic ACh receptors with 3H -QNB were determined by scintillation counting. Pieces of *Xenopus laevis* atrium were incubated with various concentrations of 3H -QNB for 1.5 h, washed in ice-cold Ringer solution for 10 h and counted. Specific binding, defined as the difference between binding in the presence and absence of 100 nM unlabelled QNB or 1 μM atropine¹³, was saturable with a single apparent K_D of 1.0 nM. Nonspecific binding in the presence of 100 nM unlabelled QNB was nonsaturable. At a saturating concentration of 2.5 nM 3H -QNB, atrial tissue contains 369 fM specific QNB binding sites per mg Lowry protein and 85 fM mg^{-1} nonspecific binding. Thus, 82% of the total binding is specific in these conditions. Autoradiographic experiments, however, show that most of the nonspecific binding occurs at the ends of muscle fibres which have been damaged or cut. Dissociation of bound 3H -QNB from receptor was determined using atrial homogenates as described by Fields *et al.*¹³. At 4°C the $t/2$ of dissociation ranged from 20 h to >48 h in five experiments. As tissues processed for autoradiography were washed at 4°C for less than 10 h not more than 20% of the bound 3H -QNB dissociated during the washing.

Muscarinic ACh receptor distribution was determined by autoradiography of whole mounts of *Xenopus* interatrial septum and sinus venosus. These tissues were chosen because they are very thin sheets, usually not more than 20 μm thick and are thus natural tissue sections¹⁵.

Figure 1 is a micrograph of a portion of interatrial septum stained with zinc iodide and osmium¹⁶ (ZIO) which stains axonal

varicosities black. The diffuse nature of the pattern of innervation is evident. ZIO-stained varicosities measure $1.05 \pm 0.45 \mu m$ in the short dimension by $1.44 \pm 0.48 \mu m$ in the long dimension (mean $\pm$ s.d., $n = 70$). Thus, an average varicosity has a cross-sectional area of $4.8 \mu m^2$. As the density of varicosities on the muscle fibres is $\sim 0.016 \mu m^{-2}$ in the interatrial septum

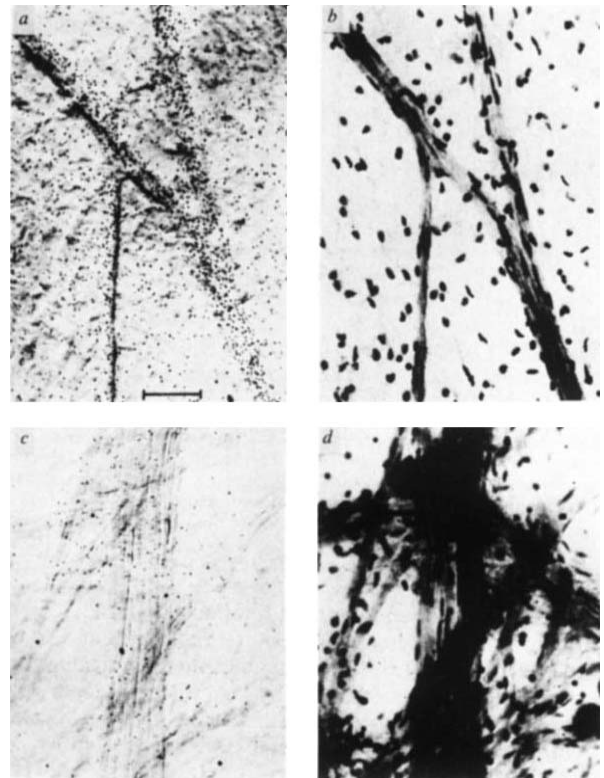


Fig. 2 *a*, Autoradiograph of a fibre in *Xenopus* interatrial septum labelled with 3H -QNB. The septum was incubated in 2.5 nM 3H -QNB (NEN, 29.4 Ci $mmol^{-1}$) in frog Ringer solution for 1.5 h, washed with numerous changes of ice-cold Ringer for 2 h, fixed for 30 min in 1% glutaraldehyde buffered with sodium phosphate, pH 7, and washed in Ringer for an additional 7 h. The thinnest portions of the preparation were cut out, placed on a microscope slide with a drop of ice-cold 2% bovine serum albumin in distilled water and rapidly dried in a stream of air at 4°C. A thin layer of carbon was evaporated onto the slide and the slide coated with emulsion by a method similar to that described by Lane *et al.*¹⁷. *b*, Same field as *a* stained with toluidine blue to illustrate cellular composition of the septum. The N-shaped strands are septal myocardial fibres which are embedded in a network of connective tissue cells whose nuclei stain darkly. *c*, Autoradiograph of a preparation incubated in 2.5 nM 3H -QNB and 100 nM unlabelled QNB to demonstrate nonspecific binding. *d*, Same field as in *c* stained with toluidine blue. Autoradiograms were exposed for 14 d. These fields are representative of seven preparations. Scale bar, 50 μm .

(manuscript in preparation), it is estimated that at most 8% of the muscle surface is synaptic; since some of the varicosities are located several μm from the muscle, focal synaptic area is probably less than 8%.

Figure 2*a* is an autoradiograph of a muscle fibre labelled with 3H -QNB in the interatrial septum. Silver grains are distributed over the entire surface of the muscle fibre. Most fibres in a given preparation exhibit a similar grain density, although occasional fibres are seen which have few grains. Silver grains are seen at much lower density over non-muscle tissue including connective tissue, endothelial cells and neurones. As the high density of silver grains is localised to the muscle fibres, little or no diffusion

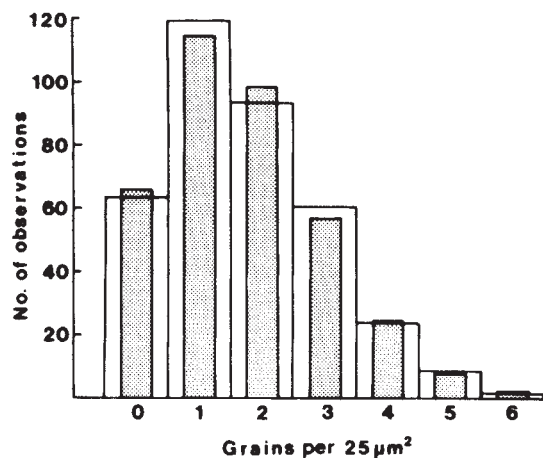


Fig. 3 Frequency distribution of silver grains of ^3H -QNB-labelled fibre. The number of $25\text{-}\mu\text{m}^2$ areas containing different numbers of silver grains are shown as a histogram (open bars). Data from cell shown in Fig. 2a. Average grain density is 1.72 grains per $25\text{-}\mu\text{m}^2$. This value was used for calculating the expected Poisson distribution (stippled bars). The calculated χ^2 value was 1.02. Similar results were obtained using unit counting areas of $10\text{-}\mu\text{m}^2$ and $100\text{-}\mu\text{m}^2$.

of QNB during autoradiography appears to have occurred. Preparations which are incubated in ^3H -QNB in the presence of 100 nM unlabelled QNB have grain densities which are not different from background (Fig. 2c) except near the ends of cut fibres.

As silver grains are produced by random radioactive decay, a uniform distribution of QNB binding sites on the fibre would show as a random distribution of silver grains in the autoradiograph. To determine whether silver grains were randomly distributed, grains were counted in $25\text{-}\mu\text{m}^2$ areas of the muscle fibre. A frequency-grain count histogram was constructed and compared to the Poisson distribution expected if grain distributions were random. Figure 3 shows that grain distribution closely approximates a Poisson process (χ^2 goodness of fit shows that the observed and expected distributions are not statistically different ($P < 0.02$)).

Muscarinic ACh receptors in the heart function to decrease the force and rate of spontaneous contraction. To be effective, therefore, the action of ACh must extend over several cycles of the beat. Purves¹⁸ has pointed out that the slow kinetics of the muscarinic response is well suited for temporal summation and continuity of ACh action over several cardiac cycles. Further, I propose that the effective modulatory influence of ACh requires spatial continuity of ACh action; that is, because probably every cell in the sinus venosus and atrium can be a pacemaker cell, focal application of ACh to a small region would be ineffective in modulating overall heart rate or force. The diffuse pattern of innervation and uniform distribution of ACh receptors is ideally suited for spatial continuity of ACh action. The arrangement of presynaptic release sites is such that transmitter is in effect 'bath applied' to the muscle and the target responds to transmitter over its entire surface. Also, the slow time course of ACh action may be due in part to diffusion of transmitter across the receptor-populated surface of the muscle.

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1. Miledi, R. *J. Physiol., Lond.* **151**, 24–30 (1960).
2. Harris, A. J., Kuffler, S. W. & Dennis, M. J. *Proc. R. Soc. B* **177**, 541–553 (1971).
3. Roper, S. J. *J. Physiol., Lond.* **254**, 455–473 (1976).
4. Fambrough, D. M. & Hartzell, H. C. *Science* **176**, 189–191 (1972).
5. Fertuck, H. C. & Salpeter, M. M. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1376–1378 (1974).
6. Kuffler, S. W. & Yoshikami, D. J. *J. Physiol., Lond.* **244**, 703–730 (1975).
7. Grillo, M. A. *Pharmac. Rev.* **18**, 387–399 (1966).
8. Burnstock, G. in *Smooth Muscle* (eds Bulbring, E., Brading, A. W. & Tomita, T. O.) 1–69 (Edward Arnold, London, 1970).
9. Hillarp, N. A. *Acta physiol. scand.* **46**, Suppl. 157, 1–38 (1959).
10. Trautwein, W. & Uchizono, K. Z. *Zellforsch.* **61**, 96–109 (1963).
11. Maekawa, M., Nohava, Y., Kawamura, K. & Hayashi, K. in *Electrophysiology and Ultra-structure of the Heart* (eds Sano, T., Mizuhira, V. & Matsuda, K.) (1967).
12. Thamer, J. C. J. *Cell Biol.* **29**, 156–162 (1969).
13. Fields, J. Z., Roeske, W. R., Morkin, E. & Yamamura, H. I. *J. biol. Chem.* **253**, 3251–3258 (1978).
14. Yamamura, H. I. & Snyder, S. H. *Molec. Pharmac.* **10**, 861–867 (1974).
15. McMahon, U. J. & Kuffler, S. W. *Proc. R. Soc. B* **177**, 485–508 (1971).
16. Maillet, M. *Trab. Inst. Cajal Invest. biol.* **54**, 1–36 (1962).
17. Lane, M. A., Sastre, A., Law, M. & Salpeter, M. M. *Dev. Biol.* **57**, 254–269 (1977).
18. Purves, R. D. *Nature* **261**, 149–151 (1976).

Progesterone derivative binds to cardiac ouabain receptor and shows dissociation between sodium pump inhibition and increased contractile force

DIGITALIS and related steroid glycosides exert two well established actions: augmentation of the contractile force of cardiac muscle, the basis for the clinical use of these drugs; and inhibition of Na-K-ATPase, a ubiquitous membrane enzyme concerned with maintenance of transcellular ionic gradients. There is much evidence to support the disputed view that the inotropic effect of the cardiotonic steroids is a direct result of Na-K-ATPase inhibition^{1,2}. In a previous study³ a large number of steroids, drugs and other substances were screened for potential activity in a radioreceptor assay for cardiac glycosides. High affinity saturable binding of ^3H -ouabain to a particulate fraction from dog heart was highly specific and antagonised only by cardioactive glycosides or their aglycones and certain steroids related to hydroxyprogesterone. The most potent progestins active in the binding assay shared certain structural features: a 17α -acetoxy moiety and unsaturation or substitution in the B ring. Of the 17α -acetate progestins available for testing in the radioreceptor assay, chlormadinone acetate (CMA), 3,5-diene-3-one- 17α -ol-6-chloro-pregn-17-acetate, was the most active. In this report we describe results of studies with CMA designed to ascertain other potential digitalis-like actions. Digitalis-sensitive processes include ion and sugar flux in rat hemidiaphragm⁴ and guinea pig atrium⁵ and Na-K-ATPase activity in several tissues⁶.

In the radioreceptor assay CMA is about 1/50th as potent as ouabain in displacing specifically bound ^3H -ouabain (Fig. 1). (If inorganic phosphate is used in place of ATP + Na^+ in the assays, CMA is about 1/20th as potent as ouabain.) Several related progestins, medroxyprogesterone acetate, megestrol acetate, and cyproterone acetate were 4–10 times less potent, whereas progesterone and 17α -hydroxyprogesterone acetate were about 100 times less potent than CMA. All other steroids tested, including 17α -hydroxyprogesterone and a large number of 17α -substituted pregnanes, androstanes and estranes, were inactive.

In both beating and resting atria, concentrations of CMA ($0.5\text{--}5.0 \times 10^{-5}\text{ M}$) markedly increased internal Na^+ , indicating inhibition of the Na^+ pump (Fig. 2). Lower concentrations had a slight stimulatory effect on the pump, reflected in lower Na^+ levels. Changes in internal K^+ (not shown) were reciprocal to those in Na^+ concentrations. The same biphasic pattern has been described for ouabain in cardiac^{5,6} and skeletal muscle^{4,7}. The biphasic effect of CMA on the influx of the non-metabolised glucose analogue, 3-O-methyl-D-glucose (Fig. 2) was also

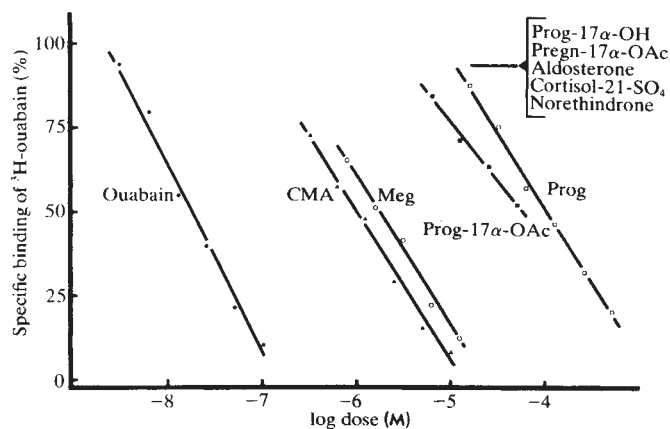


Fig. 1 Competitive binding of steroids in a ^3H -ouabain (11 Ci mmol^{-1} , Amersham) radioreceptor assay. *a*, Chlormadinone acetate (Lilly); *b*, megestrol acetate; Prog: progesterone; Preg: pregnenolone. Fresh or frozen heart ventricle from adult male dogs was minced with scissors and 20 g homogenised in a Polytron for 1.5 min in 160 ml of 10% sucrose in 10 mM Tris-HCl buffer, pH 7.4. The homogenate was filtered through a $210 \mu\text{m}$ nylon mesh screen and centrifuged at $110,000g$ for 30 min. The pellet was suspended in 160 ml of sucrose-Tris buffer, stirred for 1 h at 4°C and recentrifuged as above. The pellet was resuspended in buffer and used immediately or stored frozen in convenient aliquots. The buffer is slightly modified from that used by Schwartz *et al.*⁹ and contains 150 mM NaCl, 1 mM EDTA, 1.25 mM MgCl_2 and 50 mM Tris-HCl buffer (pH 7.4); ATP to yield a final concentration of 1.25 mM was added just before use. Competing test substance or unlabelled ouabain in 0.1 ml was mixed with 0.1 ml ^3H -ouabain in buffer to yield 9 nM of label, 0.6 ml buffer and 0.2 ml of freshly prepared or frozen suspension equivalent to 12.5 mg tissue wet weight and incubated at 37°C for 60 min with shaking. Incubation was terminated by rapid cooling of the assay tubes in an ice bath and centrifuging at $2,700g$ for 20 min. The resulting supernatant containing unbound ^3H -ouabain was discarded by aspiration. The membrane pellet was dissolved in 0.3 ml of 2M KOH by heating in a 70°C water bath for 10 min, and 0.2 ml transferred to a vial containing 10 ml of scintillation medium for ^3H counting. Steroids were insoluble in the buffer and were dissolved and diluted in ethanol. In the latter case incubation medium was added to the tube and vortexed after evaporation of the ethanol under a nitrogen stream. Unlabelled ouabain was added in the same way. Reproducibility of results with actively competing steroids indicated that the compounds were taken up by the buffer phase. Identical results were obtained in a few experiments where the alcohol stock solution was added directly to the incubation medium.

analogous to the effect of ouabain in resting atria reported previously^{5,6}. Sugar transport in resting muscle has been shown to vary in parallel with the intracellular level of sodium⁴⁻⁶ which governs Ca^{2+} influx by sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchange, and to reflect, therefore, the activity of the Na^+ pump. However, sugar transport is also affected by Ca^{2+} fluxes accompanying muscular contraction⁸ and depends on the rate and force of myocardial contraction. Accordingly, in beating atria the inotropic effect of ouabain is paralleled by a large rise in sugar transport⁶ (Fig. 2, broken line) which peaks at the concentration causing maximal inotropic effect ($0.5\text{--}1.0 \times 10^{-6} \text{ M}$). In contrast, CMA elicited no such contraction-dependent rise, and its effect on sugar transport in beating atria was like that in resting muscles and paralleled the changes in internal Na^+ . The effects of CMA on sugar transport are thus consistent with a lack of inotropic effect, together with a digitalis-like effect on the sodium pump.

In microsomes from guinea pig brain, CMA inhibited Na-K-ATPase , but not Mg-ATPase , 95% inhibition occurring at $4 \times 10^{-4} \text{ M}$. CMA was tested for potential inotropic activity on six isolated guinea pig atria at concentrations from 10^{-9} to $5 \times 10^{-5} \text{ M}$. In no instance was there any increase in contractile force; at high concentrations a decrease in contractility was

usually noted. In contrast, ouabain at $5 \times 10^{-7} \text{ M}$ consistently enhanced contractile force severalfold, the effect becoming apparent within 2 min and increasing over the 3-min observation period. In Fig. 3 typical records show that CMA neither exerted an action of its own on the beating heart nor interfered with the inotropic action of ouabain. In a separate series of experiments, cyproterone acetate also failed to affect contractility but consistently inhibited the pump.

Thomas *et al.*¹¹ reported a lack of strict proportionality between Na-K-ATPase inhibition and cardiotoxic activity of

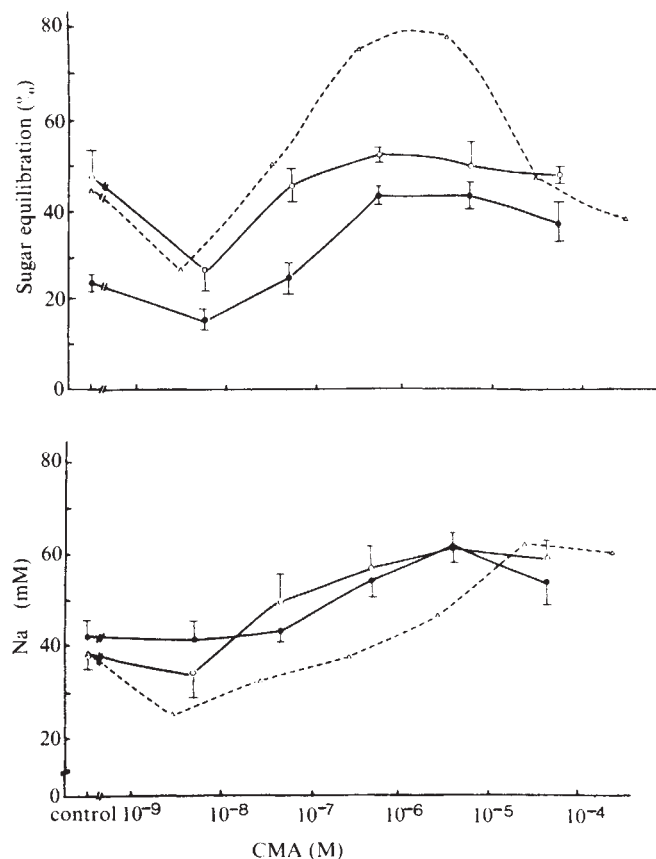


Fig. 2 Effect of CMA on internal Na levels and sugar transport in guinea pig atria. ●, Resting atria; ○, beating atria, 75 min^{-1} . Isolated guinea pig left atria were prepared and perfused as described¹⁰ with ^{14}C -labelled 3-O-methyl-D-glucose (5.0 mM), tracer amounts of ^3H -inulin and CMA in the concentrations indicated on the abscissa. The ordinates show, respectively, per cent equilibration of 3-methylglucose in the intracellular water space, reached after 15 min perfusion, and intracellular concentration of Na^+ , determined by flame photometry, at the end of perfusion; both values were corrected for inulin space. Tissues were exposed to CMA for 15 min before and during perfusion with sugar. Stock solution of CMA in 95% ethanol was added dropwise to the buffer containing 4% 'fatty acid-free' serum albumin (Sigma). Concentrations of CMA in the buffer up to 10^{-4} M yielded clear solutions which were then used to replace the total volume of the organ bath. The final ethanol concentration was 0.13% and appropriate vehicle controls produced no detectable effects. Radioactivity was determined by liquid scintillation spectrometry¹⁰. Vertical bars indicate $\pm \text{s.e.m.}$ For comparison, effects of ouabain in beating atria are included (Δ) (redrawn from ref. 6). Statistical analysis (Student's *t* test) sugar transport: increased in beating against resting ($P < 0.01$) at all concentrations up to $5 \times 10^{-7} \text{ M}$ CMA; decreased at $5 \times 10^{-9} \text{ M}$ against control in beating ($P < 0.02$) and resting ($P < 0.001$). Increased at $5 \times 10^{-7} \text{ M}$ against $5 \times 10^{-9} \text{ M}$ in beating ($P < 0.005$), and against $5 \times 10^{-9} \text{ M}$ or control in resting ($P < 0.001$). Na^+ content: increased against control at $5 \times 10^{-8} \text{ M}$ ($P < 0.05$) and all higher concentrations ($P < 0.001$) in beating and at $5 \times 10^{-7} \text{ M}$ ($P < 0.02$) and $5 \times 10^{-6} \text{ M}$ ($P < 0.001$) in resting. No significant difference between resting and beating at any concentration.

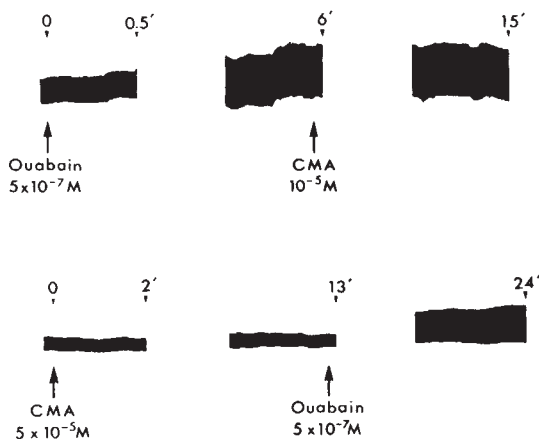


Fig. 3 Contractility of the isolated guinea pig atrium as affected by ouabain and CMA. The left atrium of male guinea pigs was suspended (resting tension 1.5 g) at 37°C in a 10-ml bath containing Krebs-bicarbonate buffer, pH 7.4, bubbled with 95% O₂/5% CO₂, and isometric contractions recorded on a polygraph with the use of a Grass force-displacement transducer. The atrium was stimulated at 2–3 Hz, 15 V, pulse duration 0.2 ms. CMA was added to the bath in a few μ l of ethanol, dimethyl sulphoxide, or Cremophore El (Sigma) with appropriate controls. The steroid was also solubilised in albumin-containing medium, in which CMA consistently inhibited the sodium pump.

some digitaloid derivatives. Our findings with CMA extend this to demonstrate complete lack of cardiotonic activity in a compound which significantly inhibits Na-K-ATPase in broken cells and Na⁺ pumping in intact cells and, moreover, competes with ouabain for specific binding sites. The data on sugar transport, serving as indicator of Na⁺ pumping and contractile activity, further support this dichotomy. These results indicate that sodium pump inhibition, even of the highly specific 'digitaloid' type, does not necessarily lead to increased contractility of cardiac cells. Furthermore, pump inhibition by CMA presumably occurs as a consequence of binding to the digitalis acceptor sites on the enzyme. This conclusion is based on the observation that CMA can displace practically all of the specifically bound ³H-ouabain, including, presumably, that portion of the cardiac glycoside which binds to and inhibits Na-K-ATPase.

The failure of CMA to antagonise the cardiotonic action of digitalis suggests that the progestin does not compete with digitalis at receptor sites coupled to events leading to increased contractility. Thus, only a very small proportion of digitalis specifically bound to cardiac membranes seems to be concerned with cardiotonic functions. It is proposed that digitalis binds with high affinity and high specificity to certain species of binding acceptor sites, also accepting active progestins, and including sites on Na-K-ATPase. In addition, digitalis may combine with another site, having little or no affinity for the progestins but concerned with the inotropic response.

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1. Akera, T. *Science* **198**, 569–574 (1977).
2. Schwartz, A., Lindenmayer, G. E. & Allen, J. C. *Pharmac. Rev.* **27**, 3–134 (1975).
3. Kim, R. S., Chow, E., Queen, G. & LaBella, F. S. *Proc. Can. Fedn Biol. Soc.* **21**, 156 (1978).
4. Bihler, I. *Biochim. biophys. Acta* **163**, 401–410 (1968).
5. Bihler, I. & Sawh, P. C. *J. molec. cell. Cardiol.* **7**, 345–355 (1975).
6. Bihler, I. & Sawh, P. C. *J. molec. cell. Cardiol.* **11** (in the press).
7. Bihler, I. in *Recent Advances in Studies in Cardiac Structure and Metabolism* Vol. 4 (ed. Dhalla, N. S.) 209–216 (University Park Press, Baltimore, 1974).
8. Neely, J. R., Liberman, H. & Morgan, H. E. *Am. J. Physiol.* **212**, 815–822 (1967).
9. Schwartz, A., Matsui, H. & Laughter, A. H. *Science* **160**, 323–325 (1968).
10. Sawh, P. C. & Bihler, I. *Can. J. Physiol. Pharmac.* **54**, 708–713.
11. Thomas, R., Rowtagy, J. & Gelbart, A. *J. Pharmac. exp. Ther.* **191**, 219–231 (1974).

Do laser diffraction studies on striated muscle indicate stepwise sarcomere shortening?

ILLUMINATION of striated muscle with a laser beam and measurement of the ensuing diffraction angles is a widely used method for the determination of sarcomere lengths. Pollack *et al.*¹ recently presented results showing that during internal shortening of single muscle fibres the angle of the diffracted first order beam changes in stepwise fashion. As the most likely explanation for this finding they suggested that the majority of the sarcomeres in the field illuminated by the laser beam, after having started shortening, suddenly cease shortening for several milliseconds before continuing the shortening process. The authors point out that this possibility would impose serious complications on the cross-bridge theory as presently envisaged^{2,3}, and in fact, on any proposed molecular theory of contraction. On the basis of diffraction studies using an experimental arrangement similar to that of Pollack *et al.* we now propose that the steps in the records of sarcomere length during contraction are an expression of slight differences in sarcomere length existing in myofibrillar clusters within the muscle fibre and therefore do not conflict with present contraction theories.

The intensity of light diffracted by striated muscle is strongly dependent on the angle ω between the beam of incident light and the fibre axis. This can be shown by rotating a single frog skeletal muscle fibre around an axis perpendicular to the fibre axis and perpendicular to the axis of a 100- μ m wide HeNe laser beam (Spectra Physics Model 155, $\lambda = 0.6328 \mu\text{m}$). During this rotation we recorded the intensity of diffracted light for each order at the respective diffraction angles using a photodiode (PIN 25, UDT Inc.). Figure 1 presents results from such ω -scans (from +15° to -25° at a speed of 2° per s) obtained with an anterior tibial fibre (diameter $\approx 120 \mu\text{m}$) at striation spacings set to a mean of 2.0 μm (a) and 2.6 μm (b). The intensity of light diffracted in the first order to the left is represented by dotted lines, and that diffracted to the right by solid lines. Such spectra show a distribution of light intensity over a range of some 20° with a halfwidth of about 10°. Sharp lines are superimposed on the continuous intensity distribution. The number and position of these lines is different for each illuminated fibre site. In general, maximum intensity does not occur at normal light incidence ($\omega = 0^\circ$). This intensity behaviour of diffracted light can be explained with the assumption that even a single fibre is too big to act as a plane grating. Rather volume effects seem to determine the diffraction process⁴.

The A-I-band pattern of adjacent myofibrils is arranged in register so that segments of high and low refractive index provide the fibre with a three-dimensional lattice of one-dimensional periodicity. Light microscopy shows that the register is not perfect in two respects. First, the segments (or idealised: lattice planes) may be inclined at a small angle η against the plane normal to the fibre axis. Second, this angle of inclination is not the same throughout the fibre diameter and along the illuminated fibre length. The myofibrils are rather arranged in clusters of variable size whose planes are inclined at small angles to each other. Thus, the lattice of the fibre resembles that of an imperfect crystal.

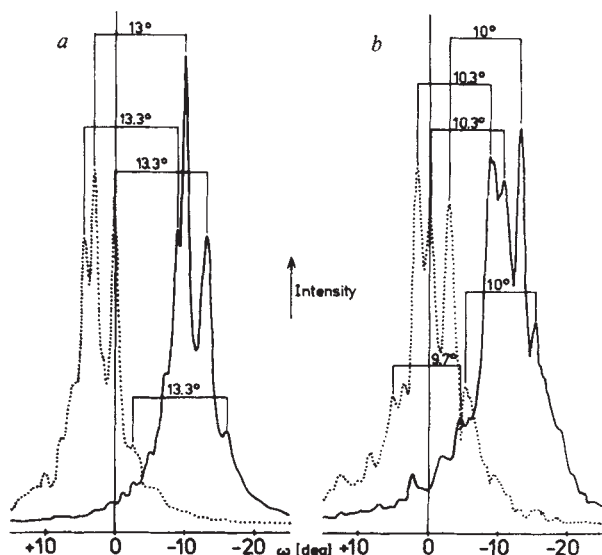


Fig. 1 Intensity of light diffracted in the first order to the left (dotted lines) and to the right (solid lines) as a function of the angle ω between incident laser beam and muscle fibre axis (linear scales, relative units). Sarcomere length set to $2.0 \mu\text{m}$ in *a* and to $2.6 \mu\text{m}$ in *b*.

Light diffraction by an imperfect volume lattice can be described using the Bragg formalism⁵. Only those lattice planes contribute to the diffraction process for which the condition $2d \sin \theta = k\lambda$ is fulfilled (d , lattice constant $\approx$ sarcomere length; θ , glancing angle between incident light and lattice plane; k , order of diffraction, λ , wavelength of light). Therefore, with normal light incidence the diffracted light is not generated by all myofibrils illuminated by the laser beam, but only by the small proportion whose planes happen to be inclined against the fibre axis at the Bragg angle ($\eta = \theta$). The Bragg formalism allows the diffraction process to be regarded as if the incident light is 'reflected' from the lattice planes, and each set of planes is able to reflect the light to the left or right if the glancing angle is appropriately set. This is why spectra of corresponding left and right orders from the same illuminated field are very similar in shape. Corresponding lines from the same myofibrillar cluster can easily be correlated as in Fig. 1. The Bragg condition requires that their positions in a plot of intensity against ω be separated by twice the Bragg angle. From the separation of the line positions, therefore, the sarcomere length of the corresponding myofibrillar cluster can be calculated.

The above points have been extensively discussed in an earlier paper⁴, but we now present evidence that the separation of corresponding lines is not exactly the same for all lines in a left-right pair of spectra. The most likely explanation for this is that different myofibrillar clusters in the fibre do not possess the same sarcomere length. As indicated in Fig. 1*a*, the lines with the largest amplitude are 13° apart, corresponding to a sarcomere length $d = 2.03 \mu\text{m}$, while most other line pairs are 13.3° apart, corresponding to $d = 1.99 \mu\text{m}$. Similarly, at the longer sarcomere length in Fig. 1*b*, the line separation varies between 9.7 and 10.3° , corresponding to $d = 2.72$ and $2.56 \mu\text{m}$. In other fibres similar variations were found, and in general the variation was at least $0.1 \mu\text{m}$ at $d = 2.5 \mu\text{m}$. Variability of the sarcomere length can also be determined from the angular dispersion of diffracted beams and variations of the same order of magnitude (2–3%) have been reported^{6,7}. These variations were, however, so far assumed to be distributed over the illuminated fibre volume in a more or less random way. We stress that the diffracted beams when viewed on a frosted glass screen show a microstructure, as described by Cleworth and Edman⁶, as if composed of several beams which show little dispersion, but

which are diffracted at slightly different angles. This microstructure and the results obtained from ω -scans (Fig. 1) suggest that sarcomere length dispersion stems from differences in the sarcomere length in the various myofibrillar clusters which themselves have a much more homogenous sarcomere length.

When a muscle fibre shortens, the Bragg condition requires that left and right hand spectra move apart along the ω -axis (Fig. 1*b* $\rightarrow$ *a*). The spectra also change their shape in an unpredictable way because different myofibrillar clusters will happen to fulfill the Bragg condition as sarcomere length decreases. If during such shortening the incident beam is first diffracted by a cluster with shorter sarcomere length and at a later time by a cluster with longer sarcomere length, the length signal in a diffractometer will behave as if the fibre ceases shortening or even lengthens, although both clusters shorten. This is schematically indicated in Fig. 2 in which for the sake of clarity this effect is exaggerated. Further, the figure represents only two clusters, one initially of $d = 2.2 \mu\text{m}$ and with an inclination η of 8.3° , the other of $d = 2.4 \mu\text{m}$ and with $\eta = 7.9^\circ$. The Bragg condition requires $\theta = 8.3^\circ$ for $2.2 \mu\text{m}$ and $\theta = 7.6^\circ$ for $2.4 \mu\text{m}$. Thus, only the shorter cluster can diffract and the diffractometer indicates $2.2 \mu\text{m}$. When both clusters shorten by $0.1 \mu\text{m}$ leaving their inclination η unchanged, at the new sarcomere lengths the Bragg condition requires $\theta = 8.7^\circ$ for $d = 2.1 \mu\text{m}$ and $\theta = 7.9^\circ$ for $d = 2.3 \mu\text{m}$. Only the longer cluster can now diffract and the diffractometer indicates $2.3 \mu\text{m}$, that is, a lengthening, although both clusters have shortened. In a sarcomere length-time plot such as those recorded by Pollack *et al.*¹ such transitions could appear as a cessation of shortening. The duration of this cessation is about 4 ms in their records. This would correspond to a difference in sarcomere length between clusters of about $0.05 \mu\text{m}$ and therefore is well within the range of differences that we found by rotating the fibre (Fig. 1). If the Bragg condition is fulfilled initially by a cluster with greater sarcomere length and later by one with smaller d , a transition will appear as an acceleration. Accelerated shortening also seems present in the records of Pollack *et al.*

Since for a given angle of laser beam incidence (such as $\omega = 0^\circ$) the first order beams to the left and right are diffracted by completely different myofibrillar clusters, transitions from one cluster to the other during shortening should be uncorrelated when recorded with the left and right first order beams. We have repeated the experiments of Pollack *et al.* with an apparatus described previously⁸, recording internal shortening alternately with the left and right beam (Fig. 3). Results from five subsequent twitches are superimposed to show reproducibility. Although the diffraction process used for the records in Fig. 3*a* and *b* occurs in the same fibre volume, the left beam consistently

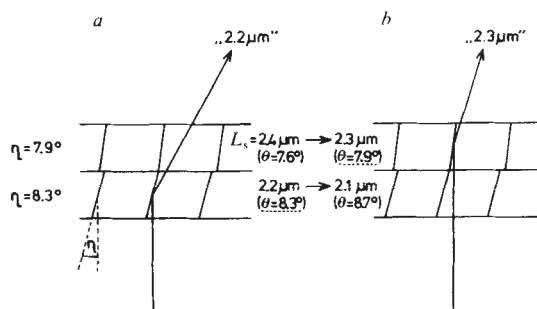


Fig. 2 *a*, Schematic drawing of a muscle fibre having two myofibrillar clusters of different sarcomere length 2.2 (lower) and $2.4 \mu\text{m}$ (upper). The striations have inclinations η against planes perpendicular to the fibre axis of 8.3° (lower) and 7.9° (upper). Only the lower cluster fulfils the Bragg condition (for $\lambda = 0.63$), therefore the diffractometer indicates a sarcomere length of $2.2 \mu\text{m}$. *b*, Both clusters shorten by $0.1 \mu\text{m}$ leaving their inclinations unchanged. Now only the upper cluster fulfils the Bragg condition, therefore the diffractometer indicates $2.3 \mu\text{m}$. For clarity all angles are doubled in the drawing.

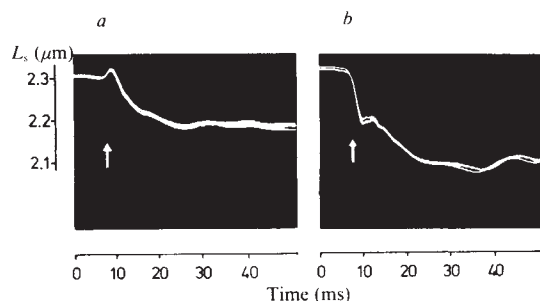


Fig. 3 Records of the time course of sarcomere length during internal shortening of a frog semitendinosus fibre. Panels *a* and *b* were obtained from exactly the same fibre volume recording the left (*a*) and right (*b*) first order beam. Traces obtained from five subsequent twitches are superimposed.

indicates a lengthening while the right beam indicates an accelerated shortening at the same time, 8 ms after the stimulus (arrows).

Although our experiments do not exclude the possibility of stepwise sarcomere shortening, they allow an interpretation of the stepwise changes during shortening of the angle of the diffracted beams which does not impose complications on molecular theories of contraction. The results suggest that the determination of sarcomere length from diffraction angles is not as simple and unproblematic as often believed. In particular, the sarcomere length determined at $\omega = 0^\circ$ may not be at all representative for the majority of the illuminated sarcomeres.

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1. Pollack, G. H., Iwazumi, T., terKeurs, H. E. D. & Shibata, E. F. *Nature* **268**, 757–759 (1977).
2. Huxley, H. E. *Science* **164**, 1356–1366 (1969).
3. Huxley, A. F. *J. Physiol., Lond.* **243**, 1–43 (1974).
4. Rüdel, R. & Zite-Ferenczy, F. *J. Physiol., Lond.* **290** (in the press).
5. Bragg, W. L. *Proc. R. Soc.* **189**, 248 (1913).
6. Cleworth, D. R. & Edman, K. A. P. *J. Physiol., Lond.* **277**, 1–17 (1972).
7. Kawai, M. & Kuntz, I. D. *Biophys. J.* **13**, 857–876 (1973).
8. Zite-Ferenczy, F. & Rüdel, R. *Pflügers Arch.* **374**, 97–100 (1978).

Evidence for the low molecular weight nature of scrapie agent

THE nature of the aetiological agent of scrapie disease, a transmissible encephalopathy naturally affecting sheep and goats, remains a perplexing problem of fundamental biological importance. Recent studies on brain tissue from outbred Syrian hamsters infected with a strain originating from the Chandler strain of mouse-adapted scrapie have led to a report of a DNA component essential for scrapie infectivity as suggested by sensitivity to DNase¹. The data presented here describe a purification procedure resulting in scrapie infectivity which migrates during polyacrylamide gel electrophoresis as a defined area of infectivity in a region characteristic of low molecular weight nucleic acid. This electrophoretically migrating species of the scrapie agent retains the characteristic disease properties by intracerebral inoculation of crude hamster brain homogenates. Infectivity isolated from this region was resistant to treatment with a combination of SDS and proteinase K followed by

ethanol precipitation. These data support the suggestion that the scrapie agent may be an infectious low molecular weight DNA.

Bioassay and electron microscopic examination of post-ribosomal high speed supernatant (HSS) subcellular fractions from hamsters clinically affected with scrapie disease revealed substantial amounts of scrapie infectivity and an absence of discernible membrane structures². Furthermore, 10% of that infectivity remained in the supernatant fraction after centrifugation at 100,000g for 44 h in sucrose-free buffer. Many of the properties of the scrapie agent—thermal stability, resistance to UV irradiation, nucleases, and proteinases—have been attributed to the intimate association of the scrapie agent with host cell components³. Accordingly, scrapie infectivity in the relatively less complex HSS fractions was more sensitive to heat and UV irradiation than in membrane-rich fractions⁴. However, exposure of the HSS fractions to the more specific degradation of nucleases and proteinases resulted in no apparent loss of infectivity. These results suggest that while discernible membrane structures were no longer associated with the scrapie agent, complexes might still offer protection of scrapie infectivity.

In an attempt to partially purify scrapie infectivity from host-cell components, the HSS was sequentially precipitated with 20, 40, 60 and 80% ammonium sulphate. Bioassay data indicated that scrapie infectivity was associated with all precipitated fractions and that the scrapie specific activity was not increased with respect to protein (Table 1). However, similar procedures applied to purified nucleic acids suggested that precipitation with 20 or 40% ammonium sulphate might result in preparations with low concentrations of host nucleic acids (Table 2), thus increasing scrapie specific activity with respect to cellular nucleic acid.

The 0–40% ammonium sulphate precipitate was electrophoresed on cylindrical 2.2% polyacrylamide gels containing 0.5% agarose and 0.1% SDS for 2 h at 6 mA per gel⁵. Absorbance profiles at 260 and 280 nm were identical when either scrapie-infected or uninfected samples were electrophoresed (Fig. 1*b*). Bioassay of eluates from gel sections indicated that a significant fraction of the scrapie infectivity penetrated the gel matrix and migrated with a characteristic mobility (Fig. 1*c*). Scrapie infectivity from gel eluates has been shown to be resistant to degradation by RNases and proteinases but sensitive to DNases with a decrease in infectivity (LD_{50} per ml) of three log units¹. As the hamster–scrapie bioassay system is only sensitive to changes in 90% of the total infectivity resulting in a change of one log₁₀ unit, concentration of three log₁₀ units of scrapie infectivity in one region of the gel over that found in other gel regions indicated a substantial level of purification by electrophoresis.

These results suggest that scrapie infectivity may be associated with a population of complexes of heterogeneous size and composition, from which the putative minimal infectious unit could be released or purified by ammonium sulphate precipitation and polyacrylamide gel electrophoresis. Whether the separation of this form of the scrapie agent from the HSS was due to partitioning of an existing minimal form of the scrapie agent or to a salt-dependent release of scrapie infectivity is not yet known.

Table 1 Ammonium sulphate precipitation of scrapie infectivity from HSS fractions

Ammonium sulphate (% at 0°C)	Scrapie infectivity (log ₁₀ LD ₅₀ per ml)	Specific activity (log ₁₀ LD ₅₀ per μg protein)
0	8.7	6.4
0–20	8.0	5.9
20–40	8.0	5.1
40–60	8.0	5.6
60–80	8.0	5.1
80 supernatant	5.5	3.9

Table 2 Ammonium sulphate precipitation of purified radioactively labelled nucleic acids

Ammonium sulphate (% at 0 °C)	Percentage of acid-precipitable counts rRNA	DNA and tRNA
0-20	11%	11%
20-40	18%	12%
40-60	100%	16%
60-80	100%	29%

Electrophoresis of tRNA, rRNA and the citrus exocortis viroid (CEV) as RNA molecular weight markers and fragments from a *Hae*III digest of Φ X174 (RF) as DNA molecular weight markers indicated that RNA molecules of 23,000–70,000 and double-stranded DNA molecules of 48,000–130,000 molecular weight migrated to the region from which high scrapie activity could be eluted (Fig. 1a). The small size of the scrapie agent suggested by the electrophoretic mobility is compatible with the estimated target size deduced from inactivation by ionising irradiation^{6,7} and a small DNA eluted from hydroxyapatite⁸. Nucleic acid markers of low molecular weight migrate in a similar way to that of scrapie infectivity; it must be cautioned that the scrapie preparation may not be assumed to be a purified nucleic acid, and that association of scrapie DNA with one or more macromolecules could affect the migration pattern.

The apparent low molecular weight and increased sensitivity to degradative treatment upon purification resemble the properties of viroids⁹, the pathogenic RNA molecules responsible for several plant diseases. However, application of procedures for the extraction of the citrus exocortis viroid¹⁰ to infected brain tissue have resulted in a complete loss of infectivity¹¹. Possible explanations for the loss of infectivity are (1) that the nucleic acid must be associated with another component in order to be infectious, (2) that the nucleic acid is infectious, but only at low levels, or (3) that recovery of the infectious nucleic acid is dependent on the extraction procedure.

To examine the nucleic acid nature of the scrapie agent and the necessity of auxiliary components further, scrapie infectivity eluted from section 3 of polyacrylamide gels (Fig. 1c) was incubated with 0.5% SDS and 50 μ g ml⁻¹ proteinase K for the deproteinisation of DNA¹², followed by ethanol precipitation and bioassay. This procedure resulted in no loss of infectivity. Examination of the gel eluate fraction under the electron microscope has revealed no particles suggestive of conventional viruses. These data further support the suggestion that the agent responsible for scrapie disease may be an infectious low molecular weight nucleic acid.

However, the possibility that the infectious unit may be nucleic acid in association with another macromolecule to form a complex unlike the regular geometric structure of viruses cannot be excluded. A nucleosome-like structure composed of 140–200 base pairs of DNA in a 10–11S nucleoprotein complex might exhibit similar migration properties in polyacrylamide gel electrophoresis, as well as variable sensitivity to DNase. A model similar to the nucleosome with DNA surrounding a protein molecule might explain the resistance of scrapie infectivity to degradation by proteinases. A further parallel might be drawn to adenovirus-5 DNA which is infectious, but with a 100-fold lower efficiency of transfection than that of the native DNA-protein complex. This DNA-protein complex is excluded from entering agarose or agarose-acrylamide composite gels during electrophoresis, while pronase-treated DNA migrates characteristically¹³, as does scrapie infectivity.

Thus the electrophoretic mobility of the minimal scrapie infectious unit is characteristic of a low molecular weight molecule, possibly of a free nucleic acid. Definition of the exact size and structure must await detection of the scrapie agent by physical procedures.

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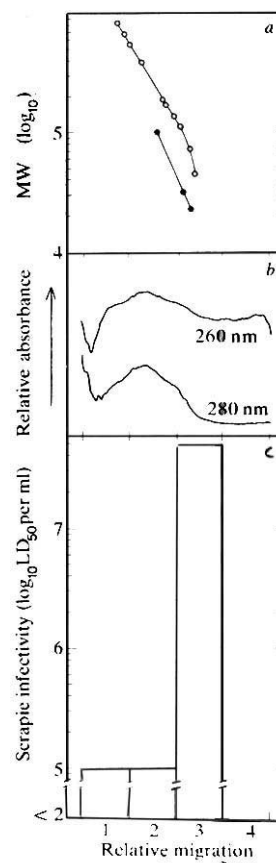


Fig. 1 Polyacrylamide gel electrophoresis of a 0–40% ammonium sulphate precipitated fraction of HSS. *a*, Migration of RNA (●) and DNA (○) molecular weight standards. *b*, Relative absorbance at 260 and 280 nm. *c*, Scrapie infectivity in eluates from 2.25-cm gel sections after the top 0.05 cm had been discarded.

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- Marsh, R. F., Malone, T. G., Semancik, J. S., Lancaster, W. D. & Hanson, R. P. *Nature* **275**, 146–147 (1978).
- Malone, T. G., Marsh, R. F., Hanson, R. P. & Semancik, J. S. *J. Virol.* **25**, 933–935 (1978).
- Millson, G. C., Hunter, G. D. & Kimberlin, R. H. in *Slow Virus Diseases of Animals and Man* (ed. Kimberlin, R. H.) (North-Holland, Amsterdam, 1976).
- Marsh, R. F., Malone, T. G., Lancaster, W. D., Hanson, R. P. & Semancik, J. S. in *Persistent Viruses Vol. II* (eds Stevens, J. C., Todaro, G. J. & Fox, C. F.) (Academic, New York, 1978).
- Bishop, D. H. L., Claybrook, J. R. & Spiegelman, S. *J. molec. Biol.* **26**, 373–387 (1967).
- Alper, T., Haig, D. A. & Clarke, M. C. *Biochem. biophys. Res. Commun.* **22**, 278–284 (1966).
- Field, E. J., Farmer, F., Caspar, E. A. & Joyce, G. *Nature* **222**, 90–91 (1969).
- Adams, D. H. *J. Neurochem.* **19**, 1869–1882 (1972).
- Diener, T. O. *Nature* **235**, 218–219 (1972).
- Semancik, J. S., Morris, T. J., Weathers, I. G., Rodorf, B. F. & Kearns, D. R. *Virology* **63**, 160–167 (1975).
- Marsh, R. F., Semancik, J. S., Medappa, K. C., Hanson, R. P. & Rueckert, R. R. *J. Virol.* **13**, 993–996 (1974).
- Gross-Bellard, M., Oudet, P. & Chambon, P. *Eur. J. Biochem.* **36**, 32–38 (1973).
- Sharp, P. A., Moore, C. & Haverly, J. L. *Virology* **75**, 442–456 (1976).

Guanine nucleotides distinguish between two dopamine receptors

BEHAVIOURAL¹ and neurophysiological² data suggest the existence of more than one dopamine receptor in the brain³. Differences in drug specificity of the dopamine-sensitive adenylate cyclase and dopamine receptors labelled by the butyrophenones, ³H-haloperidol or ³H-spiroperidol, indicate that the cyclase and the binding sites may, in part, involve distinct receptors^{4,5}. Lesion studies directly demonstrate the existence of physically distinct dopamine receptors in the corpus striatum⁶. Kainic acid microinjections, which selectively destroy intrinsic neurones in the corpus striatum, almost totally deplete the dopamine-sensitive adenylate cyclase while producing only a 40–50% decline in ³H-haloperidol or ³H-spiroperidol binding. Most of the remaining ³H-haloperidol or ³H-spiroperidol binding is lost following cerebral cortex ablation, which removes a major neuronal input to the corpus striatum, indicating that these binding sites are localised to axons and terminals of the cortico-striate projection⁶. As the dopamine-sensitive adenylate cyclase is not reduced by cerebral cortex ablation, the cortico-striate dopamine receptors do not seem to be linked to adenylate cyclase. Whether or not the ³H-butyrophenone binding sites destroyed by kainic acid represent the same dopamine receptors as those linked to adenylate cyclase has not been clear. Guanine nucleotides regulate binding at several hormone and neurotransmitter receptors, especially those associated with adenylate cyclase^{7–13}. Previously, we^{14–16} and others¹⁷ showed that dopamine receptor binding of ³H-agonists and ³H-antagonists can be regulated by guanine nucleotides. We now report that kainic acid lesions abolish the sensitivity of dopamine receptor binding to guanine nucleotides. Thus, ³H-spiroperidol and ³H-apomorphine binding sites involve two dopamine receptors, only one of which is regulated by guanine nucleotides.

After anaesthesia with Equithesin (0.6 ml, Jensen-Salisbury), Sprague-Dawley rats (160 g) were positioned in a David Kopf small-animal stereotaxic apparatus and a 0.3-mm Hamilton cannula was inserted into the striatum through a burr hole in the calvarium (coordinates: 7.9A; 2.6L; 4.8V). Kainic acid (Sigma), 2 µg in 1 µl of artificial cerebrospinal fluid titrated to pH 7.4, was infused and the scalp was then apposed with sutures. Two to five weeks after kainic acid injection, the corpus striatum ipsilateral to the lesion and the contralateral unlesioned striatum, and striata from unlesioned animals were assayed for ³H-spiroperidol and ³H-apomorphine binding, essentially as previously described¹⁴. Freshly homogenised striata (100 volumes of 50 mM Tris buffer with 0.1% ascorbic acid, pH 7.1, at 30 °C disrupted with a sonicator) were centrifuged twice at 50,000g

for 10 min with rehomogenisation of the intermediate pellet in fresh buffer. The final pellet was resuspended in buffer mix (5 mg ml⁻¹). Incubation tubes in triplicate received ³H-apomorphine or ³H-spiroperidol, tissue suspension and buffer to 1 ml. Tubes were incubated for 10 min at 37 °C and then rapidly filtered under vacuum through Whatman GF/B filters with three 5-ml rinses of ice-cold buffer. The filters were counted in 9 ml Formula 947 (NEN) after 12 h extraction at 4 °C at efficiencies of 37–44%. Saturable or specific binding was measured as the excess over blanks containing 1 µM and 10 µM *d*-butaclamol for ³H-spiroperidol and ³H-apomorphine assays respectively. Blank values were 20% and 40% of total ³H-spiroperidol and ³H-apomorphine binding, respectively.

As reported earlier, GTP lowers the binding of the agonist ³H-apomorphine with an IC₅₀ of 5–10 µM (refs 14–16) (Table 1). GDP and Gpp(NH)p (the non-metabolised analogue of GTP) have the same potency in eliciting this effect, but GMP, ATP, ADP and AMP are completely inactive.

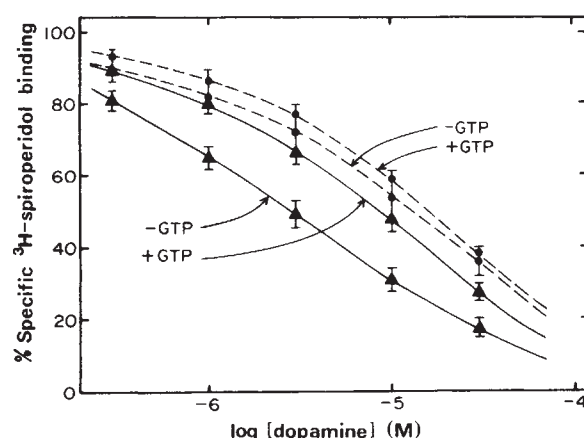


Fig. 1 The effect of striatal kainate lesion on GTP alteration of the potency of dopamine in competing for striatal ³H-spiroperidol binding. Each point is the mean of 10 determinations. Solid lines, control; dashed lines, kainate lesion.

GTP does not reduce the total binding of the antagonist ³H-spiroperidol. However, as found previously, GTP decreases the dopamine inhibition of ³H-spiroperidol binding^{14–17}. The IC₅₀ concentration for dopamine (2 µM) is increased about threefold (to 6.4 µM) in the presence of 50 µM GTP. This effect is specific in that it is exerted with the same potency by GDP and Gpp(NH)p but not by GMP, ATP, ADP or AMP. However, inhibition of ³H-spiroperidol binding by antagonists such as fluphenazine and haloperidol is unaffected by GTP¹⁶.

Kainic acid lesions, in conditions which destroy intrinsic striatal neurones as verified by biochemical and anatomical examination in this laboratory⁶, but which spare glia and neurones whose cells originate in other brain regions, reduce specific ³H-spiroperidol binding by 54 ± 3%. In lesioned animals dopamine is 4–5-fold less potent an inhibitor of ³H-spiroperidol binding than in control animals. However, the residual binding after kainate lesions still involves dopamine receptors, as drug specificity studies indicate a greater potency for dopamine than for noradrenaline or serotonin in inhibiting ³H-spiroperidol binding, and isoprenaline is inactive.

Strikingly, after kainate lesion, GTP no longer affects the potency of dopamine as an inhibitor of ³H-spiroperidol binding (Fig. 1). This loss of effect of GTP suggests that the residual binding involves a distinct dopamine receptor site which is not regulated by guanine nucleotides. To ascertain whether two such distinct dopamine receptors were labelled by ³H-agonists,

Table 1 Kainate lesion abolishes influence of GTP on striatal ³H-apomorphine binding

	³ H-apomorphine binding (pmol per g tissue wet weight)	
	Unlesioned side	Lesioned side
– GTP	18.5 ± 0.46	6.62 ± 3.2
+ 50 µM GTP	9.90* ± 0.15	5.73 ± 2.5
% Reduction by GTP	47.0 ± 1	9.0 ± 5

Kainate lesions and ³H-apomorphine binding were carried out as described in the text. Data are means ± s.e.m. for groups of 3 rats. This experiment was repeated twice in groups of 3–4 rats each. Per cent reduction data were determined by computing for each rat the ratio of binding in the presence and absence of GTP.

* Differs from unlesioned side without GTP *P* < 0.001.

we evaluated the binding of ^3H -apomorphine (Table 1). GTP (50 μM) reduces ^3H -apomorphine binding in normal rats by 47%, but no longer does so following kainate lesion, which lowers specific ^3H -apomorphine binding to 36% of control. Thus, following kainate lesions both residual ^3H -apomorphine and ^3H -spiroperidol binding is resistant to GTP.

Thus, we have found that guanine nucleotide regulation of both ^3H -agonist and ^3H -antagonist binding to dopamine receptor is lost following kainic acid lesion. This suggests that the dopamine receptors contained on the intrinsic neurones destroyed by kainic acid are regulated by guanine nucleotides, whereas those localised to the cortico-striate neurones are not. As guanine nucleotides regulate binding of receptors linked to adenylate cyclase and as the dopamine cyclase is also contained on intrinsic neurones, we conclude that the ^3H -spiroperidol and ^3H -apomorphine binding sites contained on the intrinsic neurones destroyed by kainate may involve the cyclase-linked dopamine receptors referred to as D-1 dopamine receptors^{17,18}. The notion that the cyclase-linked dopamine receptor is regulated by GTP is supported by the direct demonstration of GTP influences on the dopamine-sensitive adenylate cyclase of striatal homogenates¹⁹. By contrast, the cortico-striate dopamine receptors are not linked to adenylate cyclase and may be designated D-2 dopamine receptors.

The drug specificity of ^3H -spiroperidol binding differs from that of the dopamine-sensitive adenylate cyclase. Previously, we proposed that ^3H -agonists and ^3H -butyrophenone antagonists label distinct agonist and antagonist preferring states of dopamine receptors⁴. We suggest that ^3H -spiroperidol and ^3H -apomorphine binding to intrinsic striatal neurones destroyed by kainate label distinct agonist and antagonist preferring states of the cyclase-linked dopamine receptors. This would account for the finding that the drug specificity of the ^3H -apomorphine binding sites resembles that of the dopamine-sensitive adenylate cyclase more closely than that of ^3H -spiroperidol binding¹⁴.

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1. Cools, A. R. & van Rossum, J. M. *Psychopharmacologia* **45**, 243–254 (1976).
2. Siggins, G. R., Hoffer, B. J. & Ungerstedt, U. *Life Sci.* **15**, 779–792 (1976).
3. Keabian, J. W. & Calne, D. B. *Nature* **277**, 93–96 (1979).
4. Creese, I., Burt, D. R. & Snyder, S. H. in *Handbook of Psychopharmacology* Vol. 10 (eds Iversen, L. L., Iversen, S. D. & Snyder, S. H.) 37–89 (Plenum, New York, 1978).
5. Keabian, J. W. *Life Sci.* **23**, 479–484 (1978).
6. Schwarcz, R., Creese, I., Coyle, J. T. & Snyder, S. H. *Nature* **271**, 766–768 (1978).
7. Rodbell, M., Drans, H. M. J., Pohl, S. L. & Birnbaumer, L. *J. biol. Chem.* **246**, 1872–1876 (1971).
8. Lin, M. C., Nicosia, S., Lad, P. M. & Rodbell, M. *J. biol. Chem.* **252**, 2790–2792 (1977).
9. Mukherjee, C. & Lefkowitz, R. J. *Molec. Pharmac.* **13**, 291–303 (1977).
10. Lefkowitz, R. J., Mullikin, D., Wood, C. L., Gove, T. B. & Mukherjee, C. *J. biol. Chem.* **252**, 5295–5303 (1977).
11. Blume, A. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1713–1717 (1978).
12. Childers, S. R. & Snyder, S. H. *Life Sci.* **23**, 759–762 (1978).
13. U'Prichard, D. C. & Snyder, S. H. *J. biol. Chem.* **253**, 3444–3452 (1978).
14. Creese, I., Prosser, T. & Snyder, S. H. *Life Sci.* **23**, 495–500 (1978).
15. Creese, I. & Snyder, S. H. *Eur. J. Pharmac.* **50**, 459–461 (1978).
16. Creese, I., Usdin, T. & Snyder, S. H. *Molec. Pharmac.* (in the press).
17. Zahniser, N. R. & Molinoff, P. *Nature* **275**, 453–455 (1978).
18. Creese, I. & Snyder, S. H. in *Catecholamines: Basic and Clinical Frontiers* (eds Usdin, E., Kopin, I. J. & Barchas, J. D.) (Pergamon, Oxford, in the press).
19. Clement-Cormier, Y. C., Parrish, R. G., Petzold, G. L., Keabian, J. W. & Greengard, P. *J. Neurochem.* **25**, 143–149 (1975).

New type of twisted mesophase in jellies and solid films of chiral 12-hydroxyoctadecanoic acid

SOME optically active substances are known to form a unique state of matter, the twisted mesophase, in which a supramolecular helical structure gives rise to some striking optical phenomena¹. This type of structure was first found in melts of single compounds and later in solutions of some biopolymers. The only lyotropic twisted mesophases reported so far are the cholesteric (twisted nematic) type. However, we describe here a new type, found in the thermally-reversible jellies formed by chiral 12-hydroxyoctadecanoic acid (12HOA) with CCl_4 or aromatic solvents; solid films obtained by drying the jellies on quartz supports retained the twisted structure. Microscopy and circular dichroism (CD) indicated that this mesophase had a supramolecular helical structure, and X-ray examination of the dried solid films indicated that it was not a nematic type, but probably smectic.

Crystalline samples of 12HOA were prepared as described previously². Solutions of chiral 12HOA in CCl_4 set to an almost transparent jelly above 13 mmol l^{-1} at 20 °C, while solutions of racemic 12HOA showed no gelation until they deposited crystals from a saturated solution. The thin preparations of the jellies observed under crossed polarisers in a polarising microscope exhibited a birefringent pattern with a spherulitic or Schlieren texture of the sort observed for some liquid crystals. This characteristic appearance suggests that the jellies are a lyotropic mesophase.

The jellies exhibited a weak but definite CD in a range of wavelengths in which they absorbed no light, and the sign was negative for the D-enantiomer and positive for the L-enantiomer, irrespective of solvents used. Figure 1 shows a typical

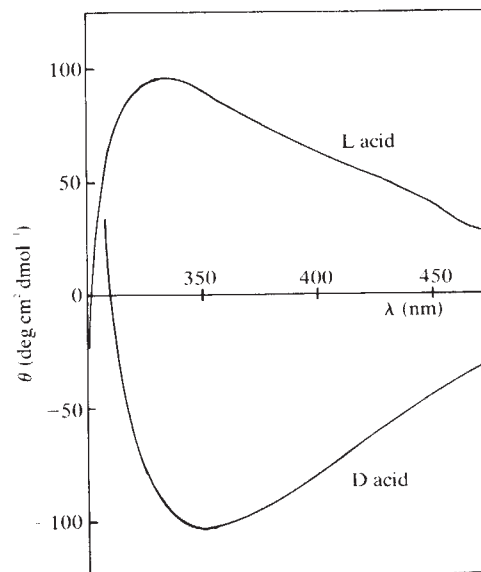


Fig. 1 Enantiomeric CD bands of jellies of 12HOA- CCl_4 (25.7 mmol l^{-1} for the L acid, 35.7 mmol l^{-1} for the D acid). These spectra were measured using a 10 mm-thick cell on a JASCO J-20A spectropolarimeter at room temperature. The location of the CD maximum was dependent on solvents (for example 350 nm for CCl_4 , 480 nm for benzene), but insensitive to change of temperature. The intensity and the location of the peak varied slightly for different samples.

example of the enantiomeric CD bands for jellies of D- and L-12HOA. Little linear dichroism was observed when jellies were examined by the procedure of Tunis-Schneider and Maestre³. Therefore we ascribed the observed CD to the preferential reflection of circularly polarised light of one sense by the jellies. The same optical phenomenon¹ has been observed for cholesteric liquid crystals and explained by assuming a supramolecular helical structure with the twist determined by the chirality of the constituent molecules.

Enantiomeric induced CD (ICD) could also be observed for some achiral molecules dissolved in jellies of chiral 12HOA. Figure 2 shows the ICD bands (superimposed on the CD curve of the jelly) and electronic spectra between 330 and 395 nm for anthracene dissolved in a jelly of D-12HOA. Interestingly, the ICD bands show a single sign and follow closely the electronic spectral bands; such ICD characteristics have been obtained for lyotropic cholesteric mesophases of poly- γ -benzyl-L(or D)-glutamate (PBG)⁴ and other polypeptides, and more recently for those of *N*-acylamino acids⁵.

In view of these CD and ICD results, it is possible that a supramolecular helical structure exists in jellies of 12HOA. Then the 350 nm CD band maximum would correspond to the 'pitch band' and the observed ICD would be caused by the dissymmetrical field of the helical structure, although it is weak compared with the effect in cholesteric systems⁴. This proposed helical structure is associated with the jelly structure in which hydrogen bonding is involved⁶, because melting of the jelly by heating resulted in the loss of all the optical properties mentioned above.

We found that all the optical phenomena described here manifested themselves more clearly in thin semi-transparent solid films (about 50 μm) obtained by drying jellies of chiral 12HOA on quartz supports. When the films were dried, the CD increased in intensity and the peak was shifted to the shorter wavelength side. These results indicate that the original supramolecular helical structure in jellies is fully retained in such solid films. Similar behaviour has been found with the cholesteric mesophase of polypeptides⁷.

Microscopy with a controlled hot stage showed that, when such solid films were heated, the mesomorphic texture became obscure at about 348 K, and then needle-like microcrystals formed: CD was no longer observed. Finally the microcrystals melted at 353 K (melting point of chiral 12HOA).

X-ray diffraction patterns for these solid films obtained by drying were similar to those for the crystalline powder, although slightly more broad. A long spacing of 46.7 Å was obtained and interpreted as the distance between double plane layers of amphiphilic molecules of 12HOA. This suggests that the mesophase structure retained in these solid films, and possibly also the structure of the mesophase itself, is not nematic, as has been shown for the cholesteric mesophase of PBG, but smectic. In the layer structure with a twisting, the molecular long axes would have to be tilted relative to the layer planes. The length of the crystallographic *c* axis of octadecanoic acid which is an analogue of 12HOA, that is, the extended length of the bimolecule, has been reported to be 49.38 Å for the B form⁸ and 50.7 Å for the C form⁹. These values are greater than 46.7 Å. This result supports the tilt possibility. It was impossible, however, to prepare orientated samples of jellies and solid films which exhibited regularly-spaced retardation lines or iridescent colours like those observed for the cholesteric mesophase of polypeptides. It seems reasonable to consider that the twisted mesophase structure exists locally as small domains which disperse with randomly-orientated helical axes within jellies or solid films. This would explain the small size of the observed CD band compared with the effect in cholesteric mesophases.

Electron microscopy has shown that both chiral 12HOA (ref. 2) and PBG (ref. 9) formed by precipitation from certain solvents produce helically twisted fibrils with handedness determined by the chirality of the molecule. Because biologically important molecules are essentially chiral, it may be biologically significant that some chiral molecules form helically

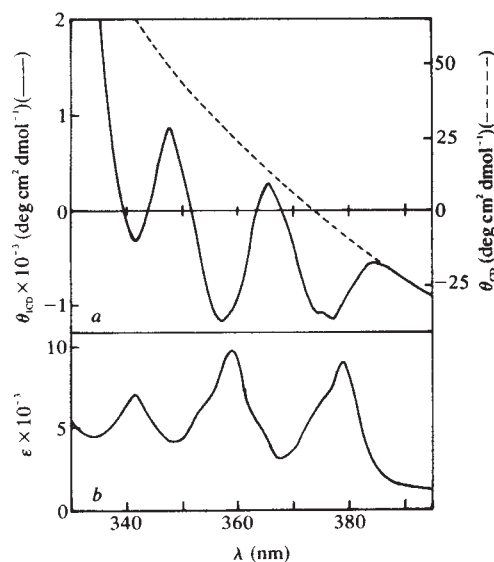


Fig. 2 *a*, ICD spectral bands (—) for anthracene (8.8 mmol l⁻¹) superimposed on the CD curve (-----) of the jelly (D-12HOA-CCl₄). *b*, Electronic spectra of anthracene. Enantiomeric ICD bands were obtained for a jelly of the L acid. On addition of anthracene, the CD maximum of the jelly itself was shifted to the longer wavelength side (~450 nm).

organised systems in different stages, as has been shown for 12HOA and polypeptides.

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1. Saupe, A. in *Liquid Crystals and Plastic Crystals* Vol. 1 (eds Gray, G. W. & Winsor, P. A.) 18-47 (Ellis Horwood, Chichester, 1974).
2. Tachibana, T. & Kambara, H. *Bull. chem. Soc. Jap.* **42**, 3422-3425 (1968).
3. Tunis-Schneider, M. J. B. & Maestre, M. F. *J. molec. Biol.* **52**, 521-541 (1970).
4. Saeva, F. D. & Olin, G. R. *J. Am. chem. Soc.* **95**, 7882-7884 (1973).
5. Sakamoto, K., Yoshida, R., Hatano, M. & Tachibana, T. *J. Am. chem. Soc.* **100**, 6898-6902 (1978).
6. Taniguchi, Y. & Suzuki, K. *J. phys. Chem.* **78**, 759-761 (1974).
7. Samulski, E. T. & Tobolsky, A. V. in *Liquid Crystals and Plastic Crystals* Vol. 2 (eds Gray, G. W. & Winsor, P. A.) 175-198 (Ellis Horwood, Chichester, 1974).
8. von Sydow, E. *Acta crystallogr.* **8**, 557-560 (1955).
9. Malta, V., Celotti, G., Zannetti, R. & Martelli, A. F. *J. chem. Soc. B*, 548-553 (1971).
10. Tachibana, T. & Kambara, H. *Kolloid-Z. Z. Polymere* **219**, 40-42 (1967).

Erratum

In the letter 'Maximum density of random placing of membrane particles' by L. Finegold and J. T. Donnell, *Nature* **278**, 443-445, the bottom line of the legend to Table 1 should be included in the table under 'Circular particles'.

Corrigendum

In the letter 'Deficient production of tyramine and octopamine in cases of depression' by M. Sandler *et al.*, *Nature* **278**, 357-358, the units of the third column of Table 2 should read nmol instead of μmol .

matters arising

Origin of the Metazoa

THE recent article by LaBarbera¹, in which the late Precambrian appearance of multicellular organisms is related to the development of shallow-water marine environments late in the Proterozoic, requires some examination of the evidence from which his conclusions have been drawn. LaBarbera bases his arguments on Hargraves² model for the evolution of the Earth through the Precambrian. Hargraves' model has been shown by Windley³ to be totally incompatible with the geological evidence as it relates to processes taking place on the Earth's surface.

LaBarbera's statement that "during the first three-quarters of the Precambrian . . . , shallow water environments (defined here as less than 200 m depth) were very rare, and those that did exist were the result of volcanic or dynamic mountain-building processes and were geologically transitory" cannot stand unchallenged.

While it can be argued that, apart from the 3,000 Myr Pongola sequence⁴, most shallow-water environments recognised in Archaean sediments⁵⁻⁹ may have been geologically transitory, it cannot reasonably be disputed that from about 2,500 Myr ago, shallow-water environments occurred frequently, were developed extensively and were geologically persistent. Examples are too numerous to list here¹⁰, but a few outstanding and clearly documented cases from the Early and Middle Proterozoic from widely separated areas are the sediments of the Athapuscow Aulacogen¹¹ and Kilohigok Basin¹² of Canada, the Transvaal Basin of South Africa¹³, and the Nabberu¹⁴ and McArthur Basins^{15,16} of Australia. All the major Proterozoic basins contain stromatolitic carbonate sediments, most of which are interpreted as being of shallow-water marine origin¹⁷. Thus LaBarbera's comment that the shallow-water fossils "are restricted to a surprisingly few localities" cannot be sustained. Furthermore, these basins frequently contain additional sedimentological evidence which, taken together, indicates deposition in shallow-water environments; for example, numerous breaks in sedimentation, with desiccation, formation of evaporites¹⁵, oolitic, pisolitic and intraclastic beds, and

tidal channel and associated continental marginal deposits. In fact, in the Proterozoic record, it is deep-water sediments that are rare, and not shallow-water ones.

A further point which must be challenged is LaBarbera's contention that early life evolved in deep-water environments, and that its most primitive manifestations must have been planktic. However, the assemblages¹⁸ which he quotes as being the "fossil record for most of the Precambrian" are all associated with stromatolitic carbonates, and they are all benthic. The organisms lived at the sediment-water interface, or just below it, and trapped or precipitated carbonate sediments by their metabolic activities. Some of the described microfossils may have had a planktic phase in their life cycle, or isolated species (for example, *Eosphaera*¹⁹ from the Gunflint Iron Formation, Canada) may have lived in surface waters—but in all cases, the water depth was shallow to very shallow. Clear evidence for oceanic microplankton appears only in the latest Precambrian.

Two recently described assemblages from Middle Proterozoic shales^{20,21} contain large spheres that may be planktic; but petrographic and palaeogeographic studies show these shales to be products of shallow-water environments. It should be added, however, that oceanic plankton has poor preservation potential in deep water unless it has very chemically resistant walls²².

Thus the major point that LaBarbera had hoped to demonstrate for the evolution of multicellular organisms is geologically untenable. A number of other recent models²³⁻²⁷ are more compatible with the geological evidence.

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1. LaBarbera, M. *Nature* **273**, 22–25 (1978).
2. Hargraves, R. B. *Science* **193**, 363–371 (1976).
3. Windley, B. F. *Nature* **270**, 426–428 (1977).
4. von Brunn, V. & Mason, T. R. *Sediment. Geol.* **18**, 245–255 (1977).
5. Ramsay, J. G. *Trans. geol. Soc. S. Afr.* **LXVI**, 354–401 (1963).
6. Lowe, D. R. & Knauth, L. P. *J. Geol.* **6**, 699–724 (1977).
7. Henderson, J. B. *Can. J. Earth Sci.* **12**, 1619–1630 (1975).

8. Dunlop, J. S. R. *Archaean Cherty Metasediments: their Sedimentology, Micropalaeontology, Biogeochemistry, and Significance to Mineralisation* Vol. 2, (eds Glover, J. E. & Groves, D. I.), 30–38 (Geol. Dept and Extension Service, Univ. West Australia, 1978).
9. Barley, M. E. *Archaean Cherty Metasediments: their Sedimentology, Micropalaeontology, Biogeochemistry, and Significance to Mineralisation* Vol. 2, (eds Glover, J. E. & Groves, D. I.) 22–29 (Geol. Dept and Extension Service, Univ. West Australia, 1978).
10. Cloud, P. E., Jr. *Annex. Trans. geol. Soc. S. Afr.* **1**–33 (1976).
11. Hoffman, P. F. *Geol. Surv. Can. Pap.* **73-1** (A), 151–156 (1973).
12. Cecile, M. P. & Campbell, F. H. A. *Bull. Can. Petrol. Geol.* **26**, 237–267 (1978).
13. Truswell, J. & Eriksson, K. A. *Precamb. Res.* **2**, 277–303 (1975).
14. Hall, W. D. M. & Goode, A. D. T. *Precamb. Res.* **7**, 129–184 (1978).
15. Walker, R. N., Muir, M. D., Diver, W. L., Williams, N. & Wilkins, N. *Nature* **265**, 526–529 (1977).
16. Plumb, K. A. & Derrick, G. M. *Economic Geology of Australia and Papua New Guinea* (ed. Knight, C. L.) Vol. 1, 217–252 (Aust. Inst. Min. Metall., Melbourne, 1976).
17. Walter, M. R. (ed.) *Stromatolites*. Vol. 20, 1–790 (Elsevier, Amsterdam, 1976). N.B. 1046 references to occurrences of Proterozoic stromatolites.
18. Schopf, J. W. *Precamb. Res.* **5**, 143–173 (1977).
19. Barghoorn, E. S. & Tyler, S. A. *Science* **147**, 563–577 (1965).
20. Peat, C. J., Muir, M. D., Plumb, K. A., McKirdy, D. M. & Norvick, M. S. *BMJ. Aust. Geol. Geophys.* **3**, 1–17 (1978).
21. Horodyski, R. J. & Bloeser, B. *Science* **199**, 682–684 (1978).
22. Muir, M. D. *Archaean Cherty Metasediments: their Sedimentology, Micropalaeontology, Biogeochemistry, and Significance to Mineralisation* Vol. 2 (eds Glover, J. E. & Groves, D. I.) (Geol. Dept and Extension Service, Univ. West Australia, 1978).
23. Towe, K. M. *Proc. natn. Acad. Sci. U.S.A.* **65**, 781–788 (1970).
24. Schopf, J. W., Haugh, B. N., Molnar, R. I. & Satterthwaite, D. F. *J. Paleont.* **47**, 1–9 (1973).
25. Stanley, S. M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1486–1489 (1973).
26. Glaessner, M. F. *Biol. Rev.* **37**, 467–494 (1962).
27. Valentine, J. W. & Moores, E. M. *J. Geol.* **80**, 167–184 (1972).

LABARBERA REPLIES—Muir, Walter and Jackson (and others¹) have apparently misinterpreted the purpose of my article² despite the explicit statement in the first paragraph that it was an "attempt to evaluate some of the implications of Hargraves' model of the geophysical evolution of the Earth during the Precambrian for the evolution of the metazoan phyla". The statement cited by Muir *et al.* concerning the existence of shallow-water environments through much of the Precambrian was not my conclusion, although failure to quote the entire sentence from my article makes it sound as though it were (missing from their quote is the phrase, "The primary implication of Hargraves' model is that . . .").

Muir *et al.* take two other quotes out of context, and by doing so change their original meanings. The complete relevant section reads, "The fossil record for most

of the Precambrian is restricted to algal stromatolites and associated microfossils which are acknowledged to be of shallow water origin. However, these fossils are restricted to a surprisingly few localities...'. Nowhere do I either state or imply that Precambrian deep-water deposits are common in an absolute sense or more common than Precambrian shallow-water deposits. Nor, as is obvious from the full quote, do I deny the shallow-water origin of stromatolites. The point of this section (perhaps too tersely made) was that one cannot presume full knowledge of the history of life on an entire planet over approximately 3,500 Myr when the fossil evidence is restricted to a single (shallow-water) environment from approximately 30 sequences³.

My article² was not a defence of Hargraves' model⁴ but an exploration of some of its implications. I welcome the contributions of Windley⁵ and Knoll⁶, who have attempted to separate fact from fancy, to this discussion. However, the extent to which Hargraves' model is applicable to Precambrian geological history is still being evaluated^{7,8} and some observations^{8,9} have yet to be adequately explained in the context of more traditional models.

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1. Henderson-Sellers, B. & Henderson-Sellers, A. *Nature* **275**, 776 (1978).
2. LaBarbera, M. *Nature* **273**, 22–25 (1978).
3. Schopf, J. W. A. *Rev. Earth planet Sci.* **3** (1975).
4. Hargraves, R. B. *Science* **193**, 363–371 (1976).
5. Windley, B. F. *Nature* **270**, 426–428 (1977).
6. Knoll, A. H. *Nature* **276**, 701–703 (1978).
7. Hargraves, R. B. *Nature* **276**, 459–461 (1978).
8. Kahn, P. G. K. & Pompea, S. M. *Nature* **275**, 606–611 (1978).
9. Saxena, S. K. *Science* **198**, 614–617 (1977).

Mutagenesis of Chinese hamster cells is facilitated by thymidine and deoxycytidine

THE presence of thymidine and/or deoxycytidine in cell cultures has been reported by Peterson *et al.*¹ to modify the mutagenic potential of methylation by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. It has long been proposed that *O*⁶-alkylguanines are potential promutagenic lesions in alkylated DNA² and this has been supported by *in vitro* experiments with bacterial RNA polymerase³. From our own *in vitro* experiments⁴ using *Escherichia coli* polymerase I acting on methylated poly(dC-dG) templates, we have concluded that any miscoding (that is, mutations, shown by the incorporation of dTMP) arising from the presence of *O*⁶-methylguanine in the template is

competitive with the normal (correct) incorporation of dCMP. Furthermore, the amount of miscoding observed depends on the relative concentrations of the DNA precursors dTTP and dCTP used in the assay. Such competitive miscoding by *O*⁶-methylguanine leads to the possibility of an alteration in the level of miscoding by factors which influence the size of the deoxynucleoside 5'-triphosphate DNA-precursor pools within the cell. It is therefore possible that agents which alter the size of these pools may enhance, or reduce, the mutagenic potential of alkylating agents. Although factors governing mutation within cultured mammalian cells are extremely complex, we believe that our observations may in part explain the results obtained by Peterson *et al.*

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1. Peterson, A. R., Landolph, J. R., Peterson, H. & Heidelberger, C. *Nature* **276**, 508–510 (1978).
2. Loveless, A. *Nature* **223**, 206–207 (1969).
3. Gerchman, L. L. & Ludlum, D. B. *Biochim. biophys. Acta* **308**, 310–316 (1973).
4. Abbott, P. J. & Saffhill, R. *Br. J. Cancer* **36**, 404 (1977); *Biochim. biophys. Acta* (in the press).

Synergistic chelation therapy or mixed ligand complexes for plutonium and cadmium poisoning?

PROGRESS in the development of chelating therapeutic drugs has been disappointing, especially when one considers the variety of diseases which respond to trace element regulation¹. The unparalleled success of two simultaneously administered ligand drugs at eliminating plutonium, reported by Schubert and Derr², is therefore most impressive.

Conceptually, the use of such double ligand treatment is not new; it has been advocated for the removal of both lead³ and copper⁴. However, Schubert's remarkable biological data emphasise the need to understand how a combination of chelating agents act. We suggest that there are alternative explanations besides the formation of mixed ligand complexes proposed in ref. 2, in which both drugs are complexed on to the metal ion.

In this context, biological transport is by passive diffusion⁵ so there are at least six requirements which a chelating agent must ideally satisfy. It should (1) bind the metal strongly enough to compete with biological ligands, especially proteins; (2) discriminate against the relatively abundant calcium and zinc cations *in vivo*; (3) be sufficiently lipophilic to penetrate membranes and hence reach the site of

heavy metal deposition; (4) form a lipophilic complex in the body compartment where the metal has accumulated; (5) change to a hydrophilic complex in plasma so the metal is eliminated in urine, not redistributed into other tissues; (6) have a low toxicity (this has implications for each of the preceding requirements).

A single ligand cannot easily meet these conditions, particularly those dealing with lipophilicity. For example, the commonly used polyaminocarboxylic acids are confined to extracellular space and rely on naturally occurring ligands to leach the poisonous metal out of the tissues^{2,5}. On the other hand, two dissimilar drugs can be chosen to satisfy collectively all the above-mentioned requirements. Synergistic chelation therapy occurs when one drug mobilises the metal while the other traps it in plasma to be excreted in the urine.

Low molecular weight transition metal complexes in biological systems almost always occur at concentrations well below the level of analytical detection⁶. Thus, we advocate that computer simulation studies of speciation in biological fluids^{2,6} be extended to plutonium and cadmium to establish whether Schubert's drugs bind to the metal ion simultaneously *in vivo* as he suggests, or whether they function separately.

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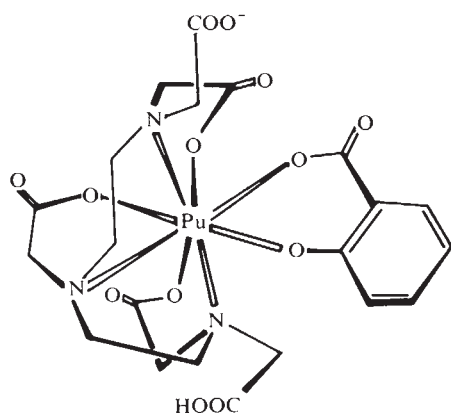
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1. Fiabane, A. M. & Williams, D. R. in *The Principles of Bio-inorganic Chemistry* (The Chemical Society, London, 1977).
2. Schubert, J. & Derr, S. K. *Nature* **275**, 311–313 (1978).
3. May, P. M. & Williams, D. R. *FEBS Lett.* **78**, 134–138 (1977).
4. Jackson, G. E., May, P. M. & Williams, D. R. *FEBS Lett.* **90**, 173–177 (1978).
5. May, P. M., Williams, D. R. & Linder, P. W. in *Metal Ions in Biological Systems* Vol. 7 (ed. Sigel, H.) 29–76 (Marcel Dekker, New York, 1978).
6. May, P. M., Linder, P. W. & Williams, D. R. *JCS Dalton*, 588–595 (1977).

SCHUBERT AND DERR REPLY—The use of double ligand therapy for decorporation of metals and therapy of metal poisoning is discussed in several references cited in our necessarily brief report. Combinations of chelating agents for decorporation were used in Hamilton's laboratory at Berkeley¹ assuming, as is usual^{2,3}, that pharmacokinetic differences would produce enhanced effects. In some instances, mixed ligand chelates which might have formed were either too weak and/or used chelants readily metabolised, such as citrates³. The results we aimed for and achieved⁴, required deliberate use of chelant combinations which form mixed ligand chelates with stabilities orders of magnitude greater than single chelants. From

the known chemistry of mixed ligand chelate formation, as we describe elsewhere⁵, the choice of suitable combinations is straightforward. For example, Martell *et al.*⁶ demonstrated, using potentiometric titrations, that Th(IV) formed very stable mixed ligand chelates with EDTA, salicylate and catechol (10^{11} – 10^{13} times more stable than EDTA alone). As expected, Pu(IV) reacted similarly.

We reject the suggestion, unsupported by experimental evidence, that alternative explanations besides mixed ligand complexes are responsible for our results. Our experiments^{4,5}, including ultrafiltration, solvent extraction, and *in vivo* reversal of salicylate antipyresis by Zn and Cu EDTA, provided experimental evidence for mixed ligand chelate formation. The proposed mixed ligand chelate of Pu with DTPA and salicylate is depicted by the following structure:



The requirements for biological transport emphasised by May and Williams have long been known. It is not necessarily true, however, that highly charged polyaminopolycarboxylic acids are confined to extracellular spaces. A very small but adequate fraction of these chelants penetrate intracellular space as shown by sustained excretion of metals following single treatment^{7,8}. Charged metal ions, for example, chromate, can also penetrate cell membranes and reach cell interiors⁹. May and Williams' proposal that synergistic chelation therapy occurs when one drug mobilises the metal and the other traps it in plasma is inapplicable to our systems, as compounds such as salicylate and catechol by themselves do not react with Pu(IV) in *in vivo* conditions.

Although computer simulation studies of speciation in biological fluids have a certain value, they are unnecessary in our therapeutic investigations, as even simple non-computer calculations readily show that the very strong mixed ligand chelates used by us bind >99% of the total metal. Indeed, this is shown in their own calculations¹⁰, even with single chelants, and computer calculations used previously¹¹ by one of us. The ability of complexing

agents to bind metals in the blood stream does not necessarily coincide with the ability to remove metals deposited in the tissues¹².

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- Schubert, J. A. *Rev. nucl. Sci.* **5**, 369–412 (1955).
- Magos, L. & Stoytchev, T. *Br. J. Pharmac.* **35**, 121–126 (1969).
- Volf, V. *Hlth Phys.* **29**, 61–68 (1975).
- Schubert, J. & Derr, S. K. *Nature* **275**, 311–313 (1978).
- Schubert, J. & Derr, S. K. *Radiat. Res.* (in the press).
- Carey, G. H., Bogucki, R. F. & Martell, A. E. *Inorg. Chem.* **3**, 1288–1295 (1964).
- Fried, J. F., Graul, E. H., Schubert, J. & Westfall, W. M. *Atompraxis* **5**, 1–5 (1959).
- Lindenbaum, A. & Schubert, J. *Nature* **187**, 575–576 (1960).
- Mertz, W. *Physiol. Rev.* **49**, 163–239 (1969).
- May, P. M. & Williams, D. R. *FEBS Lett.* **78**, 134–138 (1977).
- Sharma, V. S. & Schubert, J. *J. Am. chem. Soc.* **91**, 6291–6296 (1969).
- Schubert, J. & Rosenthal, M. W. *Am. med. Ass. Archs ind. Hlth* **19**, 169–178 (1959).

Evolution of the 3', 5' internucleotide bond

DHINGRA AND SARMA¹ posed the question "Why do nucleic acids have 3', 5' phosphodiester bonds?". They concluded from a detailed NMR investigation of the conformations of dinucleoside monophosphates that 2', 5'-linked oligomers are unlikely to form helical structures that are stabilised by base-stacking. Thus 2', 5'-linked nucleic acids would be less suitable for participation in present day biochemical operations that involve helix formation. Dhingra and Sarma considered a polymer as a set of repeating dimer structures and did not consider the perturbations that could be introduced by the presence of a complementary strand. There is, of course, evidence that 2', 5'-linked oligomers can complex with 3', 5'-linked complementary polymers, although the melting temperatures of these complexes do tend to be lower than in the case of an all 3', 5'-linked complex².

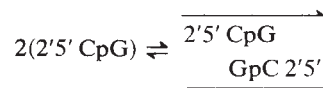
We considered this same question some years ago, and predicted³ that the 2', 5' bond in a helical oligonucleotide would be more susceptible to non-enzymatic chain breakage than would the 3', 5' bond. A large difference in kinetic stability was subsequently demonstrated experimentally for a triple helix of oligoadenylates and poly U in mildly basic solution⁴. Thus during the early chemical evolution of nucleic acids, it seems reasonable to suppose that there was a strong selective pressure for the 3', 5' bond, and against the 2', 5' bond.⁵

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- Dhingra, M. M. & Sarma, R. H. *Nature* **272**, 798 (1978).
- Michelson, A. M. & Monny, C. *Biochim. biophys. Acta* **149**, 107 (1967).
- Usher, D. A. *Nature new Biol.* **235**, 207 (1972).
- Usher, D. A. & McHale, A. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1149 (1976).
- Usher, D. A. *Science* **196**, 311 (1977).

SARMA AND DHINGRA REPLY—Since the publication of our paper in *Nature* we have carried out extensive NMR studies in 100% water on self-complementary 2', 5'-dinucleoside monophosphates, that is



to monitor the Watson-Crick hydrogen bonding protons. The data showed that 2', 5'-linked dimers have little proclivity to form Watson-Crick hydrogen bonded miniature double helices under conditions in which their 3', 5' analogues have been shown¹ to form such complexes. The study has revealed that the availability of a complementary dimer does not introduce enough perturbations to the intrinsic stereochemistry of 2', 5' systems to induce the formation of miniature double helices. In this connection, it is important to note that in the reported crystal structure of 2', 5' ApU no miniature double helices have been observed², contrary to the crystal structure findings on 3', 5' ApU and 3', 5' GpC^{3–8}, but like 3', 5' UpA⁹.

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- Shefter, E., Barlow, M., Sparks, R. A. & Trueblood, K. N. *Acta crystallogr. B* **25**, 895–909 (1969).
- Rosenberg, J. M. *et al. Nature* **243**, 150–154 (1973).
- Rosenberg, J. M., Seeman, N. C., Day, R. O. & Rich, A. J. *molec. biol.* **104**, 145–167 (1976).
- Seeman, N. C. *et al. J. molec. Biol.* **104**, 109–144 (1976).
- Hingerty, B. *et al. Biopolymers* **14**, 227–236 (1975).
- Day, R. O., Seeman, N. C., Rosenberg, J. M. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **70**, 849–853 (1973).
- Hingerty, B. *et al. Acta Cryst.* **B32**, 2998–3013 (1976).
- Seeman, N. C., Sussman, J. L., Berman, H. M. & Kim, S. H. *Nature non. Biol.* **233**, 90–92 (1971).

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

reviews

Historical cetology

J. H. S. Blaxter

The Natural History of the Whale. By L. Harrison Matthews. Pp. 219. (Weidenfeld and Nicolson: London, 1978.) £12.50.

THE *World Naturalist Series* (not to be confused with the *New Naturalist Series*) continues with a book on whales by the editor of the series. There are nine chapters—on Cetology from its Beginnings, Cetacean Diversity, Food and Feeding, Breeding and Growth, Swimming and Diving, Migration, Communication and Echolocation, Behaviour and Social Relations, Parasites, Diseases and Enemies—followed by a list of living species, a bibliography and index.

The book is very readable and is especially strong on the historical aspects of research on whales. Unlike so many subjects where the older literature tends to become obsolete, modern cetology depends very much on the earlier work. One of the reasons for this lies, alas, in the appalling over-exploitation of whales, so that many species are protected and some may be near extinction. Thus, material, often available in the past only through the whaling industry, is no longer obtainable. The discoveries made have been impressive, especially when one considers the difficulty of handling such large specimens, the problem of rapid decomposition before they can be dissected and the need, often, to play 'second fiddle' to the whale-catchers. Much of the more recent discoveries on whale physiology and behaviour come from the smaller cetaceans such as the dolphins which are fairly easy to catch and keep in captivity. The building of oceanaria has also made it possible to watch and train larger cetaceans such as the Killer Whale. Speculation about echolocation, feeding habits, buoyancy mechanisms and the problems of deep diving have come from the study of so-called functional anatomy.

This all makes fascinating reading. Everything is on a massive or record scale. The blue whale is the largest living animal; whales make migrations of 28,000–30,000 kilometres and can 'sprint' at 20 knots; sperm whales can dive to 3,000 feet and become entangled in submarine cables; the Antarctic whales in their heyday ate 150 million tons of krill annually; some

whales have tape-worms which are 50 feet long!

I found the style of the book rather uneven. The author expects a fairly high standard of knowledge from the reader when discussing some of the anatomy, especially that of the skull, yet elsewhere, for example, he discusses the genetic code, DNA and the philosophy of immortality (pages 76 and 137). In general the diagrams, though clear, are inadequately labelled and have no scale given. There are 16 pages of black-and-white plates which cannot fail to fascinate the reader. One aerial photograph of a school of white whales in the Canadian Arctic showing adults and calves is especially interesting.

The author is decidedly acerbic about some of his predecessors and of some later investigators. On page 28 he gives very short shrift to *Nessiteras rhombopteryx* P. Scott (1975) the "non-animal of Loch Ness" (reported in *Nature* 258, 466, 1975). On page 101 he castigates the owners of pets who recycled whale meat as dog-droppings on city pavements. He is rightly uncompromising in his criticism of anthropomorphism in our interpretation of cetacean behaviour. For example, the explanation of the apparent saving of

human swimmers in distress by dolphins lifting them to the surface almost certainly lies in the lifting of newly-born calves by their mothers to the surface for their first breath of air. Other instances of relationships between cetaceans and man such as the riding of ships' bow-waves can easily be explained as advantageous to the animal, which saves energy as it utilises the bow-wave to push itself along. Although the author is not in possession of the results of naval research on the use of dolphins for underwater warfare, he thinks their intelligence has been overestimated and their potential is limited. If dolphins were really intelligent would they not take every opportunity "to pull a human swimmer in difficulties down to death" considering that man has been a ruthless destroyer of dolphins for centuries?

Dr Harrison Matthews' book is quite different in style from two recent books on sea mammals reviewed in *Nature* (270, 641, 1977). It deals only with cetaceans and in greater detail. I would rate it as reading for any biologist or well-informed laymen. □

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Summary of rheological activity

Mechanics of Non-Newtonian Fluids. By W. R. Schowalter. Pp. 300. (Pergamon: Oxford, 1978.) £17.50.

THIS text, directed toward graduate students and others beginning research in rheology, begins with a fine motivational chapter 1 and then launches into a five-chapter development of mathematical and physical foundations in terms of tensor analysis. This segment, rigorous but rather dry for students, would require elaboration by an instructor to be fully successful.

The following three chapters are designed to describe rheometric and related flows; Noll's simple-fluid concept is used throughout. Chapter 7 introduces the idea of viscometric flows and provides several illustrations. The equations of continuity and motion for incompressible fluids, in the three major coordinate systems, are

appended. Measurements of the viscometric functions are described in chapter 8, in which numerous flows are studied: plane and cylindrical Couette flow, axial motion in open and annular tubes, jet methods (jet swell, thrust on tube), and the Jackson-Kaye elevated cone procedure. A survey of experimental difficulties is given separately, including end effects, viscous heating, and hole error. Chapter 9 emphasises stretch-history concepts, specialising to extensional flow and perturbations from viscometric flows.

Model-building is encountered in chapters 10 and 11. Elementary models—purely viscous, generalised Newtonian, and linear viscoelastic (differential and integral) fluids—are found in chapter 10. For the first time molecular concepts are introduced, through the dilute-solution polymer dynamics theory of Zimm. The latter treatment is

somewhat patchy and needs to be read with the five chapter appendices to be coherent. The appendices describe Gaussian springs, Brownian motion, hydrodynamic interaction, stress tensor construction, and finally full solution of the eigenvalue problem. (It is unfortunate, however, that the relaxation time τ_p is implied on p155 to represent the response of the *pth* sub-molecule rather than the *pth* normal mode.) Chapter 11 generalises this material in various ways, including a description of the Oldroyd approach and the use of non-linear time derivatives in modelling. Commentary on many of the models is so brief that a trek to the cited literature is really required.

Chapter 12 on applications comes close to several engineering problems. Again, the treatment is a survey, but this reader found it quite successful: boundary layer theory, converging flow, falling sphere, extensional motion, flow in packed beds, transients, secondary motion, stability theory, extruder analysis, orthogonal rheometer and

inclined open channel. Chapter 13 concludes the book with another excursion into the microscopic, 'Suspension Rheology'. Both rigid and deforming particles are considered, with most detail given to dilute emulsions.

Arising from notes used by Professor Schowalter in over ten years of teaching, the book contains homework problems appended to 11 of the 13 chapters and ample references. Few data are given—only three figures show data points, although there are many schematic drawings—and notation is cumbersome in places. However, the writing style is clear and contains a fine flavour of good humour, and relatively few misprints have crept in. One is advised to ignore the purple and lavender cover and use this text as a guide to the wide variety of rheological activity during the modern transition of 1950–75.

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Atomic absorption spectroscopy

Atomic Absorption, Fluorescence and Flame Emission Spectroscopy: A Practical Approach. By K. C. Thompson and R. J. Reynolds. Pp. 319. (Charles Griffin: London and High Wycombe, UK, 1978.) £15.

THE second edition of this book, first published in 1970, includes coverage, in varying detail, of the very extensive advances which have been made in atomic spectroscopy in the intervening years. In some areas, however, the extent of the coverage leaves something to be desired. There is commendable emphasis on safety, of which the reader should take careful note: unfortunately, burner explosions are not unknown.

The book can be considered in two main sections, one being principally chemical and the other instrumental. The chemical section covers very thoroughly the areas of applications, chemical techniques and interferences in atomic absorption spectroscopy (AAS), although some of the instrumental procedures described tend to be somewhat dated. For instance, the inexperienced reader would be forgiven for thinking that digital readout is a rare feature, whereas, in fact, it is incorporated in nearly all present-day instruments. It is also noticeable that strong emphasis is given to the relatively little used hydride technique,

whereas the very important area of flameless electrothermal AAS is relegated to the chapter on "Some further techniques"; and it should be mentioned that some authorities claim considerably lower detection limits for

Molecular models

Molecular and Crystal Structure Models. By A. Walton. Pp. 201. (Ellis Horwood: Chichester, UK, 1978.) £9.

MAKING models of the physical world is the stuff of science. Extending and refining these models is what we are doing when we do research. Nowhere is the concept of models more evident than in chemistry. Both at the detailed level of molecular orbitals and, more commonly, in the consideration of three dimensional structure we consider molecules to be representable by models which we can imagine actually constructing as tangible objects.

Dr Walton's book surveys most of the commercially available molecular models and contains advice on do-it-yourself versions. The survey is not exhaustive (an exhaustive survey would soon date), but it is sufficiently wide to be a useful first step for anyone looking for a particular feature.

Models showing special features or behaving in special ways are commonly constructed in research and this book will provide useful tips for that would-be modeller. It is, however, to

some elements than those given in the table on pages 33 and 34.

On the instrumental side, it seems that the authors have a bias against double-beam instruments and the reader is advised to be cautious in accepting the advice given. It is also felt that the guidance on performance parameters could be clearer for the inexperienced user. Again, it is noticeable that only brief mention is made of microprocessor-based instruments, although these now represent a high proportion of those available.

Flame emission and atomic fluorescence spectroscopy are covered in a separate chapter, primarily from the point of view of comparison with AAS. In this respect, an unfortunate weakness is the fact that the very important and competitive (with AAS) technique of plasma emission is dismissed in five lines.

A very extensive bibliography is given at the end of each chapter, but it might have been appropriate to mention that a complete atomic absorption bibliography is available. Overall, the book can be recommended to both new and more experienced users of AAS from the chemical standpoint but with reservations on the instrumental side.

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the teacher that it will most appeal, providing as it does descriptions of most of the off-the-shelf models, and photographs of many. In it one should be able to find the (non-ideal) model which is a good compromise and which can be provided within the departmental budget for a class of students.

Apart from being inexhaustive, the book suffers from, inevitably, describing each model in a different way. It is particularly unfortunate that it was not possible to have photographs of every model type. This makes it difficult to use for searching out models of a particular type, but it does make it readable. The principal deficiency is, however, the lack of information on consumer-response—is it easy for students to see the point the lecturer is making? Do the students like building these models and do they understand the principles that they are supposed to demonstrate? What are their deficiencies? Perhaps the only answer, as every teacher wishes to emphasise different aspects, is to use this book (with its extensive cross-referencing) to narrow down the choice and then try the models oneself.

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Sedimentary environments

Ancient Sedimentary Environments. Second edition. By R. C. Selley. Pp. 287. (Chapman and Hall: London, 1978). Hardback £8; paperback £4.95.

THIS is an inexpensive book, in which the essential features of the major sedimentary environments are succinctly described. In a book of 287 pages, the treatment does not attempt to be detailed and each synthesis is supported by a generally well chosen list of references. The references, which form 17% of the text, are one of the strengths of the book, for they provide either the student or the experienced geologist with basic references up to 1977. Chapters cover, sedimentary environments and facies; river deposits; wind-blown sediments; lake deposits; deltas; linear clastic shorelines; mixed clastic; carbonate shorelines; shelf deposits; carbonate and terrigenous; reefs; flysch and turbidites; pelagic deposits, and sedimentary models, mythical and mathematical. As might be expected, the emphasis reflects the author's wide field experience, but the omission of glacial sediments is surprising.

The opening chapter contains definitions and discussions of a sedimentary environment and facies. Although brief, the discussion is a clear statement of important basic concepts. For instance—"One of the main problems of determining the origin of ancient sediments is that, though essentially reflecting depositional environments, they also inherit features of earlier erosional and non-erosional phases"—a truism which gives rise to many problems in interpretation.

The author points out that it is only sedimentary structures (and palaeocurrents) which unequivocally indicate the depositional environment. The five defining parameters of a sedimentary facies—geometry, lithology, sedimentary structures, palaeocurrents and fossils—are examined in an instructive way, flavoured with many cautions derived from experience.

In many cases, environmental interpretation must be based on borehole or seismic data, so the inclusion is very appropriate of a general section on the use of vertical grain-size profiles from geophysical logs and the use of the down-hole dipmeter.

The whole text is intended as a practical guide to "environmental interpretation [which] is at best an imprecise art, rather than a deterministic scientific discipline", though the author's treatment is a good preparation for

travelling along the latter route. Generally, each chapter has a main section covering one or more case histories, followed by sections giving wider views of the particular sedimentary facies, its economic significance, subsurface recognition and finally a list of references. Numerous clearly presented diagrams reduce the need for lengthy descriptions.

By its very nature, the book is a compilation of generalisations, but these are made by a perceptive geologist who continuously reminds his readers that the exceptions are the

really interesting problems. The whole presentation is admirably clear and concise, making it a most suitable book for a second-year university course. Geologists at all levels will gain much information from the eminently readable and witty style, but the most important impact is the encouragement of a discriminating and critical sifting of geological evidence. At such an economical price, the book is a good investment.

D. Hamilton

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Physiology of fungal colonies

Practical Fungal Physiology. By P. M. Robinson. Pp. 122. (Wiley: Chichester, UK, and New York, 1978.) £3.50.

ONE can only agree with the author's opening statement in the preface to this book that "The potential of fungi as experimental material is not always fully exploited". This book provides a series of experiments designed to introduce a student to some basic aspects of fungal physiology. There are nine chapters and the suggested experiments are described in each chapter within an overall account of the cellular processes involved. Topics dealt with include spore germination, hyphal growth, fungal nutrition, morphogenetic substances, staling, colony morphology, mycostasis and continuous culture. The experiments described in most chapters are very basic and have been designed to utilise a minimum of expensive equipment. There are references to original papers in the relevant areas and figures are included to

illustrate the expected results of certain experiments. Experiments are described in flowsheet form and adequate details are provided for media recipes, inoculation times and general experimental protocols.

Overall, this simplified approach should provide a reasonable introduction to the physiology of the fungal colony. It is, perhaps, unfortunate that many of the experiments involve aspects of fungal physiology of whose cellular and biochemical mechanisms we have little understanding. This limitation is compounded by the omission of experiments involving well understood phenomena such as the phototropic responses of *Phycomyces*.

Despite these criticisms the book should prove useful to school and university teachers looking for ideas to be included in an introductory course on mycology. Also, if the book helps move the teaching of mycology away from the classical taxonomic approach, then it is to be welcomed.

Keith Gull

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Molecular dynamics

Molecular Structure and Dynamics. By W. H. Flygare. Pp. 696. (Prentice-Hall: Englewood Cliffs, New Jersey, and Hemel Hempstead, UK, 1978.) £18.25.

ONE can tell from the front leaf of the dustcover that this is a crowded treatise. There is the ^{13}C -NMR spectrum of a methyl group with its free induction decay, the ESR spectrum of the cyclopentyl radical, the Raman spectrum of CO_2 , the low resolution IR spectrum of CH_2Cl_2 , the radial wave function of the 1^1S orbital, a coordinate system for electron scattering from a triatomic molecule and another relating laboratory and molecular axes, and the X-ray structure factor for

metallic potassium. If the back leaf had been used to show the electron density difference plot of HF, the splitting pattern of a ^3D state in an octahedral field (with spin-orbit interaction), the Doppler spectrum of interstellar formaldehyde and the symmetry elements of CH_4 , then one could have dispensed with the statement of contents.

The book would be suitable for a BSc honours course in chemical physics or for a postgraduate course for spectroscopists. It is a well written book and I found little to quarrel with in either the contents or their presentation.

I must, however, admit to a preference for shorter books on a narrower front. Seven hundred pages is indigestible and I cannot envisage ever starting such a book and getting to the end. Of course, there are books on my shelf that I use just for reference, and per-

haps this will be added to their number. Professor Flygare clearly hopes for more—for example, by challenging the reader with problems at the end of each chapter.

The difficulty with an epic work is that one dare not be discursive or one cannot get it all between two hard covers. Let me take, for example, the section on scattering in this book. The fifty pages devoted to this topic is sufficient to include topics such as vibrational and rotational inelastic scattering or the relationship between the potential energy surface for a reaction and the energy distribution in the products.

Mitochondrial and plastid heredity

Organelle Heredity. By N. W. Gillham. Pp. 602. (Raven: New York, 1978.) \$64.35.

NON-MENDELIAN HEREDITY has been the Cinderella of genetics ever since Correns discovered it exactly 70 years ago. It has long been complicated, confused and experimentally intractable. It is still complicated, but powerful new techniques, ably described in this new book by one of their chief wielders, are rapidly reducing the confusion.

For over a decade it has been clear that there are two fundamentally different causes of non-Mendelian heredity. One of these, the direct inheritance of preformed cell structure—seemingly independently of DNA—is well established only for the ciliate cortex. The more widespread cause is the existence of DNA inherited other than in a Mendelian fashion. Sometimes such DNA is nuclear—B-chromosomes are the most obvious example—but more usually it is cytoplasmic. Although there are numerous scattered cases where cytoplasmic DNA constituting the genome of intracellular viruses or cellular endosymbionts causes non-Mendelian inheritance, cytoplasmic DNA occurs regularly only in plastids and mitochondria; the genetics of these, not of organelles in general, is the subject of Gillham's excellent book.

The book is the most thorough treatment yet of mitochondrial and plastid genetics, and deserves to be read widely by geneticists, biochemists, cell biologists and advanced students. Although it excludes evolutionary discussions, it will be invaluable to all interested in organelle evolution. Fifteen of the twenty-one chapters concentrate on transmission genetics, the four on baker's yeast and the four on *Chlamydomonas* forming the heart of the book.

There is no mention of the Born approximation or of semi-classical quantisation (apart from the Bohr atom). The book would not be sufficient in itself for a postgraduate course on molecular dynamics and would be a little thin even for an advanced undergraduate course on this topic.

Perhaps I am being petty. It is in the main a fine work which I shall find very useful. I doubt, however, that it will be adopted as a course book in many British universities. **J. N. Murrell**

J. N. Murrell is Professor of Chemistry at the University of Sussex, Brighton, UK.

Nuclear transmission genetics was substantially worked out within a single decade of Morgan's first concentration on *Drosophila*. Yet although Ephrussi and Sager made yeast and *Chlamydomonas* the drosophilas of cytoplasmic heredity decades ago, we still do not understand it. This is because until the past decade the characteristic uniparental inheritance of organelle genes, and the scarcity of different mutants, precluded traditional genetic analysis of segregation and recombination. Much of the recent progress stems from strictly genetic advances—the ability to obtain biparental inheritance and to induce and isolate a greater variety of mutants. This has led to an impressive array, often bewildering to the outsider, of new mapping techniques; and it is valuable to have them so clearly laid out in one place.

The discovery of organelle DNA has also been important, especially on the psychological side as a booster of the intellectual respectability of the field. Wherever possible Gillham integrates the molecular and genetic approaches, but where this cannot be done, as in the conflicting data on the inheritance of *Chlamydomonas* chloroplast DNA, he simply describes the basic data. His account of the conflicts between Sager's work and his own are admirably balanced. Particularly valuable are the comparisons between the yeast and *Chlamydomonas* work. Although yeast is simpler and more intensively studied, in the long run *Chlamydomonas* will be ideal for studying the interactions between biparentally inherited nuclear DNA, 'maternally' inherited plastid DNA and its 'paternally' inherited mitochondrial DNA. ('Maternal' and 'paternal' are peculiarly inappropriate for *Chlamydomonas* which is isogamous and has an organelle like the acrosomal tubule of sperm on its so-called 'maternal' gametes.)

Mitotic segregation prevents enumeration by the *cis-trans* test of the polypeptides coded by organelle DNA.

but genetic techniques can help in assigning specific gene products to nuclear or organelle DNA: Gillham ably describes the combined genetic and biochemical study of mutants, the use of interspecific hybridisation and the analysis of mammalian cell hybrids in tissue culture. DNA hybridisation studies of mules and hinnies have shown mitochondrial DNA to be maternally inherited, as in fungi. Here, however, the mechanism may simply be dilution of sperm mitochondria by the vast excess of female ones in the egg. In fungi, many of which are isogamous like *Chlamydomonas*, this cannot be so; curiously, twelve years after I discovered chloroplast fusion in *Chlamydomonas* the cytological fate of mitochondria in fungal zygotes is still unknown, although in 1978 Gaffal demonstrated mitochondrial fusion in zygotes of the alga *Polytoma*. As Gillham points out, cytology is lagging behind genetics and biochemistry.

Although emphasising genetics, Gillham also summarises present biochemical, cytological and physiological knowledge of the structure and functions of organelle DNA. The first chapter is an up-to-date outline of the structure and function of plastids and mitochondria. It is followed by one on the structure and replication of mitochondrial and chloroplast DNA, including brief accounts of the underlying techniques. The two chapters on the RNA- and protein-synthetic machinery of plastids and mitochondria, and their biogenesis, describe the evidence that organelle RNA is coded by organelle DNA; the majority of proteins, on the other hand, including nearly all the ribosomal ones, are coded in the nucleus and synthesised outside the organelles.

The remaining two chapters discuss the biosynthesis of the mitochondrial inner membrane and the thylakoids. Here also most of the proteins are coded by the nuclear DNA; only a handful are yet known to be coded by the organellar plasmids. I suspect that the bulk of this DNA—like nuclear DNA—does not code for proteins. The pace of advance of both the genetic and the biochemical methods Gillham so clearly describes is such that we shall soon know; things too recent to be included are constantly being discovered, like the presence of split genes in both mitochondria and plastids.

This well produced book, with its ample references, is a milestone in the history of genetics.

T. Cavalier-Smith

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